University of Alberta

EFFECTS OF INCREASED TEMPERATURE ON EPILITHON AND THE IMPLICATIONS OF CLIMATE CHANGE

by

Helen Margaret Baulch ©

A thesis submitted to the
Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
Master of Science

in

Environmental Biology and Ecology
Department of Biological Sciences

Edmonton, Alberta
Spring 2002

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"When the well's dry, we know the worth of water."

-Benjamin Franklin

Abstract

Climatic change is expected to lead to increases in surface water temperatures of many lakes. This study addressed the impact of increased water temperatures on epilithon, the biofilm associated with rocky substrata. We developed an *in situ* warming system, and heated experimental enclosures by 4.5°C for eight weeks. Increased water temperatures caused a strong change in the algal composition of an early successional community, but did not significantly affect a more mature community. Limited effects on carbon accrual, stoichiometry and invertebrates were noted, and a strong increase in bacterial abundance was observed as a result of warming. Analysis of a long-term data set showed that metabolic parameters were positively correlated with water temperature, and experimental manipulation demonstrated that increased water temperatures stimulate rates of photosynthesis and respiration. These findings, in conjunction with increased bacterial abundance indicate that littoral carbon flow may be strongly affected by increased water temperatures.

Acknowledgements

First, I would like to thank my two supervisors, Dave Schindler and Michael Turner. Dave provided me with the freedom to pursue topics of interest, and the time to find a project that I really found both interesting and important. Dave always gave solid scientific advice, but also expected me stand on my own two scientific feet; critical attributes in a supervisor. Dave also gave me the latitude to pursue a project that he wasn't sure would succeed, and provided funding despite his doubts about whether the engineering machine would ever work. Dave and Suzanne Bayley provided hospitality and conversation during much of the development stage, as testing was done in the Lobstick River, a few hundred feet from their home. And, when the water level started rising on the Lobstick, and floodwater threatened to sweep my enclosure downstream, Dave rescued it. Dave saw first hand the trials and tribulations of trying to heat water *in situ*, but was patient, helpful, and encouraging. It was Dave's outstanding reputation as a researcher, and his outspoken nature that brought me to Alberta to work with him. I have benefited from both and learned about both science and advocacy from Dave.

Michael too has been an excellent supervisor. I should first note that I came as a great surprise to Michael. Following a conversation with Elise Watkins in March or April of 2000, I contacted Michael and started to talk to him about long-term data, and experimental work. Michael was enthusiastic and encouraging about my ideas, and generously allowed me use of his long-term data set. Much of the planned long-term analysis is incomplete, but is a project I hope we will continue in the future. Michael accommodated and assisted me a great deal in the field, on short notice that I'm sure threw his plans into disarray. He provided critical review of my ideas, and helped me expand my research in ways that greatly eased the interpretation of my results, and expanded the scope of my findings. In the field Michael generously provided logistical assistance, training, the use of lab space and equipment, timely funding, and after the lightning strike that disabled the temperature control system, Michael provided chocolate. Whenever there was a logistical problem in the field, Michael would help provide a solution, and when none was immediately evident, Michael would provide me with support.

Dr. Doug Dale, Mark Ackerman and Terry Nord were critical to the technical success of this project. They helped me test a variety of temperature control systems, and persisted despite numerous failed trials in the field. Mark wrote and tested the software required for the temperature control system, and helped diagnose problems in the field. Terry designed and built the electronics required for this application. Dr. Dale created a heat budget for the endeavour, assisted in testing a temperature controller at the university, and was the person who assembled this team. When the lightning strike debilitated my equipment mid-way through the experiment, Terry spent a great deal of time with me, helping me diagnose the problems over the phone. Once the problem was identified, Terry went in to work on Labour Day to send me the package of the parts I needed to put Humpty Dumpty together again. And Terry sent a package of liquorice. Thanks Terry. You convinced me we could solve the problem, when I wasn't sure any more. All of this work was done on a very short time scale, and Terry, Mark and Dr. Dale were instrumental in helping me get into the field in the summer of 2000. These three sacrificed holidays, and tolerated phone calls at home, and I owe them a great debt in the success of this project.

Roderick Hazewinkel was the chief plumbing researcher behind this project. Rod has an amazing curiosity about him, and drive to make things work. Rod volunteered a great deal of time to help with the construction of the system. He worked with me on numerous re-designs, and was critical to the construction efforts at the ELA. Rod and I spent days at Home Depot and Revy putting pipes together on the floor, and Rod often spent lunch hours talking to staff at plumbing stores to try to find solutions to the numerous problems. Rod helped pioneer our experimental plumbing efforts, but also helped design rock-solid enclosures, and helped me laugh lots when things were going well, and when the water temperatures were out of control or air locks shut down the whole system. Rod has also been a good friend through my 2 years here, and I will miss his off-the-wall sense of humour, and getting to be a CBC geek with him over beers.

There are many others who helped with logistics. Paul Weidman helped with problem solving on site. Neil Fisher and Ian Delorme delivered propane. Bill Benson and Pat Davis provided plumbing ideas. Russell Westphal also developed a heat budget. Bob Newbury and Ian Davies helped in initial design of the heating system. Cory Matthews helped carry countless propane tanks through leech-filled waters and in perilous boats, laughing most of the time. Lyn, Stephanie, Katherine and Brenda helped lug things to boats or to my study site. And, my dad, Bill Baulch, had the fortuitous timing of arriving when things went awry, and spent a great deal of time walking back and forth with me, with flashlights, to manually control the temperature. Equipment was generously donated by Little Giant Pumps, the March Pump Company, Ipex (thanks to Doug Purdy), Superior Propane, Electro cables and CableTech.

Many members of the ELA team were involved scientifically. Len Hendzel sat in on meetings and helped in design of the exchange experiments. Paul Weidman lent his littoral experience to the project. Mark Lyng, Ken Beaty, Susan Kasian and others provided data. Paul Blanchfield provided fish advice, and Ray Hesslein has helped me with boundary layer calculations and understanding DIC dynamics. Mike Paterson kindly agreed to count time-consuming meiofauna samples and zooplankton samples. Dave Findlay counted a large number of algal samples. In addition, Rolf Vinebrooke analysed the large number of pigment samples, and has answered a great number of pigment and climate related questions. Committee members Vince St. Louis and Lee Foote provided important dialogue and guidance.

Statistical help must also be acknowledged. Drs. Prasad and Roland assisted with coding in SYSTAT, and Wendy Wright discussed analysis of the long-term data.

Members of my lab group have provided friendship, and scientific discussion the past two years. Elise Watkins entertained me during my gear up to the field season, and connected me to some of the people who have been instrumental in the success of this project. She also arrived with Rod mid-season and bolstered my spirits with music, and bowling. Suzanne Tank and I have led parallel lives in many ways, with concurrent field seasons, concurrent writing, and being neighbours. She has provided encouragement, friendship, and interesting scientific dialogue. I've enjoyed conversations with Brian Parker on a whole range of topics from political to scientific, then often back to political. Thanks also go out to Maggie Xenopoulos for advice, Paul Frost (honourary lab member) for stoichiometry dialogue and editing, Natalie McMaster for climate chats, and Michelle Bowman, Erin Kelly and Heidi Swanson for social chats. And, thanks to all for friendship.

Finally, I'd like to thank many other friends and my family. I'm not sure how to thank Chris for all of the support and love I have received. He also provided a great deal of help on the practical side, suggesting propane hot water heaters as the heat source, and spending a precious weeks holidays, including his birthday, packing, driving and building with me. Thanks Chris. New friends in Edmonton, particularly Sweaty Cheddar (Jack, Jen, Colin, Jenny, Sarah, Jason, Erik, Matt and Kelly) have been involved in many fun adventures. Old friends from home also deserve mention. I look forward to seeing more of you soon. Thanks must go to my wonderful family. My mom, dad and brother have been incredibly supportive, as have Laurie, Joyce and Marg. I have omitted many others to whom I owe gratitude – as so many have provided assistance, friendship, dialogue and support in the past two years. This project and this process have involved a very many people, and to all of them I say thanks.

Research was funded by an NSERC grant to Dave Schindler, and a Government of Canada Climate Change Action Fund grant to Michael Turner. Research at the Experimental Lakes Area is partially funded by the Department of Fisheries and Oceans. Personal funding was provided by a NSERC PGS-A scholarship, a Hammer Limnology Scholarship, a Department of Fisheries and Oceans NSERC supplement, a Canadian Water Resources Association Scholarship, a Walter H. Johns Graduate Fellowship, and an entrance scholarship from the University of Alberta.

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Chapter 1: General Introduction

Climatic effects on lakes

Lakes are strongly affected by climate via direct effects on water temperature, light, wind mixing and the length of the ice-free season. Indirect influences include effects on nutrient availability (Schindler et al., 1996a), acidity (Yan et al., 1996), water clarity (Yan et al., 1996; Schindler et al., 1996a), oxygen availability (Stefan et al., 1993), viscosity (Wotton, 1995), thermal niche space (DeStasio et al., 1996), and forest fire frequency (Stocks et al., 1998). Climatic warming is expected to affect all of these parameters. Hence predicting effects of climate change on lake ecosystems is complex.

An unusually warm and dry period at the Experimental Lakes Area (ELA) in the late 1970s and 1980s provides some indication of what the future may hold. Lakes became warmer and clearer with deeper thermoclines, and deeper euphotic zones (Schindler et al., 1990; 1996a). Water renewal time increased, as did the length of the ice-free season. Alkalinity and concentrations of nitrogen, sulfate and calcium also increased. Concentrations of silica, phosphorus, and dissolved organic carbon declined (Schindler et al., 1996a). The decline in DOC led to an increase in the depth of penetration of UVB radiation (Schindler et al., 1996b).

Modelling studies also allow us to predict possible chemical and physical effects of climate change. In addition to changes in thermocline depth (McCormick, 1990; Hondzo and Stefan, 1991; DeStasio et al., 1996), stratification may occur earlier, persist longer, and stratification strength may change (DeStasio et al., 1996; Hondzo and Stefan, 1991; 1993; Stefan et al., 1993; Stefan et al., 1996; Fang and Stefan, 1999). Hypolimnetic oxygen depletion may be more severe, and occur earlier in some lakes (Stefan et al., 1993), although problems of winterkill may be alleviated due to shorter periods of ice cover, and higher rates of under-ice photosynthesis (Fang and Stefan, 2000).

Climatic change may already be affecting the length of the ice-free season. Ice break-up has been occurring progressively earlier around the Northern

Hemisphere (Robertson et al., 1992; Assel and Robertson 1995; Anderson et al., 1996; Schindler et al., 1996; Magnuson et al., 2000).

Actual changes in lake water temperatures will depend upon the combination of changes in air temperatures, humidity, precipitation, wind, and solar radiation. In the Great-Lakes Precambrian Shield region, lake surface water temperatures during the summer are predicted to increase 1-7°C following a doubling of atmospheric carbon dioxide concentrations (DeStasio et al., 1996; Fang and Stefan, 1999). The magnitude of epilimnetic warming is expected to be greatest in April and September (Hondzo and Stefan, 1991; Stefan et al., 1993; Stefan et al., 1996). Effects of climatic change on hypolimnetic temperatures are more poorly characterized, and are strongly dependent on lake morphometry (Hondzo and Stefan, 1991; Stefan et al., 1993; 1996; Fang and Stefan, 1999). While some models predict an increase in hypolimnetic water temperatures (Stefan et al., 1996; Fang and Stefan, 1999), others predict a decrease in hypolimnetic water temperatures due to earlier and stronger stratification (Hondzo and Stefan, 1991; Stefan et al., 1996).

Temperature effects on lake biota

Temperature affects all biological reactions, and hence the importance of temperature to organisms and communities has long been recognized. Water temperature can be spatially variable, largely due to groundwater seeps, and differences in microhabitat (DeNicola, 1996). Marked seasonal and diurnal changes are also observable in lake water temperatures. However, the magnitude and rate of temperature change depends on lake volume, morphometry, water colour, bottom colour, inflows and water renewal rate (Hutchinson, 1957; Yan and Dillon, 1984).

Research into temperature effects on single species has been invaluable, and has helped elucidate a number of general trends. We know that rates of light saturated photosynthesis increase with temperature within an optimal range (Platt and Jassby, 1976; Graham et al., 1996), and rates of algal respiration also increase with temperature (Collins and Boylen, 1982; Iriarte and Purdie, 1993). In contrast, rates of light limited photosynthesis are limited by rates of photochemical reactions and are therefore considered temperature-independent (Steemann Nielsen and Jørgensen, 1968), a result corroborated by a number of studies (Post et al., 1985; Rae and Vincent, 1998). However, effects on temperature-dependent photosynthetic enzymes may lead to temperature dependence of light-limited photosynthesis (Davison, 1991), a result also supported by the literature (Levasseur et al., 1990; Coles and Jones, 2000). Algal nutrient uptake rates (Cloern, 1977; Rhee and Gotham, 1981), cellular quotas (Goldman, 1979; Rhee and Gotham, 1981; Raven and Geider, 1988; Thompson, 1999), and nutrient ratios (Thompson et al., 1992) may be temperature dependent, suggesting that food quality may vary following temperature changes. This assertion is supported by the observation that ratios of unsaturated to saturated fatty acids can decline with increased temperatures (Thompson et al., 1992). Respiration rates of invertebrates increase with temperature, as do grazing rates and fecundity (Sweeney, 1978; reviewed by Arnell et al., 1996; Höckelmann and Pusch, 2000). Age at first reproduction, development time, adult size and longevity are inversely related to temperature (Abdullahi and Laybourn-Parry, 1985; Abdullahi, 1990). Bacterial respiration rates increase with temperature (Thamdrup and Fleisher, 1998; Pomeroy and Wiebe, 2001), hence rates of organic matter decomposition also increase (Wotton, 1995).

Community studies have shown that temperature affects the outcome of competitive interactions among algae (Tilman et al., 1986; Goldman and Ryther, 1976), bacteria (Harder and Veldkamp 1971) and invertebrates (Moore et al., 1996). The observation that planktonic Cyanobacteria and chlorophytes dominate at higher temperatures than diatoms may be related to shifts in the ability of these taxa to compete for nutrients at different temperatures (Tilman et al., 1986), or may be related to changes in water viscosity and mixing (Willen, 1991). Changes in the richness and diversity of algal and invertebrate assemblages have been observed with changed temperature (Logan and Maurer, 1975; Squires et al., 1979; Oden, 1979). However, interactions among trophic levels are poorly understood. Simple predator-prey systems may be destabilized, (Beisner et al., 1997), and population turnover may accelerate at elevated temperatures. Populations of *Daphnia* and rotifers tend to turnover more quickly at increased temperatures (McCauley and Murdoch, 1987).

Rates of fish predation may increase (Elliott, 1975), and fish growth rates, assuming prey levels remain adequate, are expected to increase (Hill and Magnuson, 1990).

Interactions between direct and indirect effects of increased temperature may also be important in understanding changes in populations. For example, increased algal biomass resulting from increased temperatures may alleviate bacterial substrate limitation, and increased temperatures may ease temperature limitation of bacteria (Pomeroy and Wiebe, 2001), possibly leading to a stronger increase in bacterial activity than would be expected for either factor in isolation.

Temperature and other stressors

Interactions of multiple climate-related stressors may also be important to dictating community structure. Diatom abundance may decline as a result of increased water temperatures (DeNicola, 1996). Diatoms are also the most susceptible taxa to UVB radiation (Donahue, 2000), suggesting that climate change, which may lead to concurrent increases in penetration of UVB radiation and water temperatures, may strongly reduce diatom abundance. Similarly, increased water temperatures and UV radiation are typically associated with decreased abundance of benthic macroinvertebrates (Lamberti and Resh, 1983; Bothwell et al., 1994; Hogg and Williams, 1996). Increased water temperatures and the presence of UV radiation in a clear-cut reach of a British Columbia stream led to stronger effects on invertebrate biomass and community diversity than UV effects alone (Kelly, 2001). The projected increase in the length of the ice-free season will lead to increased annual productivity (Byron and Goldman, 1990). If stimulation of photosynthetic rates by increased water temperatures is factored in, major increases in annual photosynthesis should be expected. Increased carbon fixation, together with increased invertebrate grazing (Deason, 1980; Dumont and Schorreels, 1990), increased predation (Elliott, 1975; Dumont and Schorreels, 1990), accelerated respiration (Chapter 3), and stimulated heterotrophic activity, suggest that climate change will strongly impact carbon flow within lakes.

Study outline

This study addresses the effects of increased water temperatures on epilithon, the biofilm associated with rock surfaces. Epilithon is a heterogeneous community of algae, bacteria, fungi and invertebrates. Detritus provides much of the carbon pool within this community. Climate change responses of littoral communities, including epilithon, are poorly characterized (DeNicola, 1996) despite the importance of littoral communities to lake productivity (Wetzel, 1983) and as an energy source to higher trophic levels in some lakes (Hecky and Hesslein, 1995).

In this thesis, we analysed a long-term data set to determine whether epilithic metabolic rates were correlated with temperature. In addition, we artificially warmed enclosures to assess the influence of water temperature on epilithic metabolism and community structure. Chapter 2 describes the design of this experimental heating system. In chapter 3, the effects of temperature on epilithic metabolism are discussed, and in chapter 4 warming effects on community structure are assessed.

We experimentally manipulated water temperatures within a series of littoral enclosures in Lake 239 at the ELA. Water temperatures were increased by 4.5°C in half of our enclosures, while half were maintained as controls. This increase in water temperatures is consistent with climate change scenarios (DeStasio et al., 1996; Fang and Stefan, 1999), is within the range of annual variability in maximum epilimnetic temperature of our study lake (Schindler et al., 1996a), and is similar in magnitude to natural diurnal fluctuations (Chapter 2). The experiment was run for eight weeks to allow observation of some of the more gradually developing effects while limiting the extent of divergence of enclosures from lake conditions over time. The heating system used differential control; that is, temperatures were maintained at approximately 4.5°C above ambient, although ambient temperatures fluctuated strongly. This is important given that fecundity and development rates of invertebrates may differ under fluctuating and constant temperatures (Bradshaw, 1980).

Our objectives were: (1) to develop an experimental apparatus suitable for *in situ* temperature manipulation (chapter 2); (2) to assess temperature effects on epilithic metabolism using experimental and long-term data (chapter 3); and (3) to assess the effects of increased temperature on community composition (chapter 4).

In chapter 3, *Effects of warming on epilithic metabolism*, we tested the following hypotheses:

1) Increased temperatures will lead to increased rates of net photosynthesis as a direct result of increased temperatures (Mantai, 1974; Collins and Boylen, 1982; Blanchard and Guarini, 1997), and as a result of a shift in the algal community to species adapted to higher temperatures (DeNicola, 1996).
2) Algae will acclimate to higher temperatures by increasing their pigment content, as observed in cultures of single species (Sosik and Mitchell, 1994; Machalek et al., 1996; Coles and Jones, 2000).
3) Increased respiration rates of algae (Iriarte and Purdie, 1993; Graham et al., 1996), bacteria (Thamdrup and Fleisher, 1998; Pomeroy and Wiebe, 2001) and invertebrates (Sweeney, 1978; Höckelmann and Pusch, 2000) will lead to increased rates of community respiration in warmed enclosures.
4) Direct temperature effects will be most important to the stimulation of metabolic rates. Effects on community composition were expected to be of secondary importance.
5) Stimulation of rates of net photosynthesis will lead to increases in algal biomass.
6) Increased water temperatures will lead to an increase in the proportion of gross photosynthesis allocated to dark respiration (R_{dark}:P_{gross}) because respiration is more strongly temperature dependent than photosynthesis (Busch and Fisher, 1981).

In chapter 4, *Effects of warming on epilithon community composition*, we focussed on community level changes, testing the following hypotheses:

1) Increased water temperature will lead to increased biomass of chlorophytes and Cyanobacteria, a decline in diatoms, and a loss of algal species diversity (DeNicola, 1996).
2) A decline in mean algal cell size and bacterial cell size is expected, consistent with Atkinson's size-temperature hypothesis (Atkinson, 1994).
3) Bacterial abundance and biomass will increase with increased water temperatures, as observed in heated streams (Lamberti and Resh, 1983; Osborne et al., 1983).
4) A decline in invertebrate abundance will be observed, consistent with studies of warmed streams and lakes (Oden, 1979; Lamberti and Resh, 1983; Hogg and Williams, 1996).
5) Invertebrate species sensitive to the increase in water temperatures will decline in abundance, or disappear from warmed enclosures (Ferguson and Fox, 1978; Oden, 1979). This will lead to a decrease in invertebrate richness and diversity.
6) Warming will accelerate invertebrate development (Sarvala, 1979; Abdullahi, 1990; Wilhelm and Schindler, 2000).
7) Changes in algal nutrient uptake (Cloern, 1977; Rhee and Gotham, 1981), cellular nutrient quotas (Goldman 1979; Rhee and Gotham 1981; Raven and Geider, 1988; Thompson et al., 1992; Thompson, 1999), net photosynthesis (Phinney and McIntire, 1965), and taxonomic change (DeNicola, 1996) will lead to changes in algal nutrient ratios.
8) Carbon accrual will be greater in warmed enclosures as a result of increases in the biomass of algae and bacteria.

To determine whether community maturity affected warming responses, we studied carbon accrual, stoichiometry and algal community composition in early successional communities, and in more mature communities.

This research on metabolic and community change constitutes the first *in situ* controlled experimental temperature manipulation addressing effects of increased water temperatures on lake epilithon. This research will help further our understanding of climate effects on lake ecosystems as well as advancing our understanding of the ecological role of temperature in benthic metabolism and structuring benthic communities.

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Chapter 2: Climate change experiments: design of a mesocosm heating system

Most effects of climate change on lake ecosystems have been deduced by using models (Byron and Goldman, 1990; Regier et al., 1990), long-term monitoring (Schindler et al., 1990; George and Taylor, 1995; Güss et al., 2000), or paleoecological research (Vinebrooke et al., 1998). Studies of thermal effluents have also helped advance our understanding of the effects of increased water temperatures, but many are confounded by large increases in water temperatures, changes in the physical environment, or the presence of introduced species (Patalas, 1970; Gallup and Hickman, 1975; Klarer and Hickman, 1975; Konopacka and Jesionowska, 1995). Given current predictions of a 1.4 to 5.8°C increase in global surface temperatures between 1990 and 2100 (Intergovernmental Panel on Climate Change, 2001) and associated increases in lake temperatures, experimental work to assess the effects of increased water temperature is required. The design of this mesocosm-scale experimental technique allows *in situ* experimental manipulation with replication, and will help further our understanding of climate-change effects on freshwater ecosystems. This paper describes the design of a heating system and its performance, limitations and possible improvements.

General design

In this experiment eight 700-L enclosures were constructed within a lake. Half of the enclosures were warmed by 4.5°C above ambient water temperatures using a closed-circulation heat-exchange system. Hot water was pumped through insulated pipes to the enclosures where approximately 10m of heat exchange pipe was coiled around the inside of each enclosure bottom (1.4m x 1.4m). This system is adaptable to larger and smaller scale experiments, and can provide fixed or differential temperature control. Target temperatures or temperature differences are easily adjusted via a simple software change. In the current application, differential control was deemed important, because the lake temperatures showed strong diurnal variation, and because fecundity and development rates of invertebrates can differ under fluctuating and constant temperature regimes (Bradshaw, 1980).

Electronic temperature control

The desired experimental treatment was a temperature difference of 5°C between control and warmed enclosures. Experimental and reference enclosures were paired, and a differential thermocouple (Teflon-coated type-J thermocouple wire) was used to measure the temperature difference between the paired enclosures. Thermocouple junctions were installed near the bottom of all enclosures. Recording thermocouples (HoboTemp, Onset) were installed at the same location in all enclosures, and programmed to record water temperature every 15 minutes. Thermocouples were attached to a TFX-11 controller (Onset), and the controller was programmed (TFBasic) to produce a signal when the sensed temperature difference was less than 5°C (wiring diagram is shown in Figure 2.1). This 5V logic signal was converted to a 16 V DC signal for a relay that opened a solenoid valve (Asco electric), allowing hot water to flow into the heat exchange pipe. When the temperature difference exceeded 5°C, the controller stopped producing an output signal, and the solenoid valve closed (Figure 2.1).

The TFX-11 controller can handle up to ten channels of input and hence could be used to control temperature in ten warmed enclosures at one time. It may also be used to monitor temperatures, although the requirement of frequent downloading of data (approximately daily) precluded its use for this task in this experiment. Most importantly, temperature control was unaffected by fluctuations in air temperature. This is in contrast to two other models of temperature controllers (Chromalox Model 1601; Maxthermo MC-4501).

The initial program we tested included a dead band. Within a set temperature range, termed the dead band, the solenoid valves would not be opened or closed. This was designed to extend the lifetime of the valves. The concern was that if enclosure temperature remained near a threshold of 5°C, solenoid valves would continually cycle and eventually fail. In practice this was unnecessary. Solenoid valves did not cycle excessively. A threshold system was used for the experiment that opened the

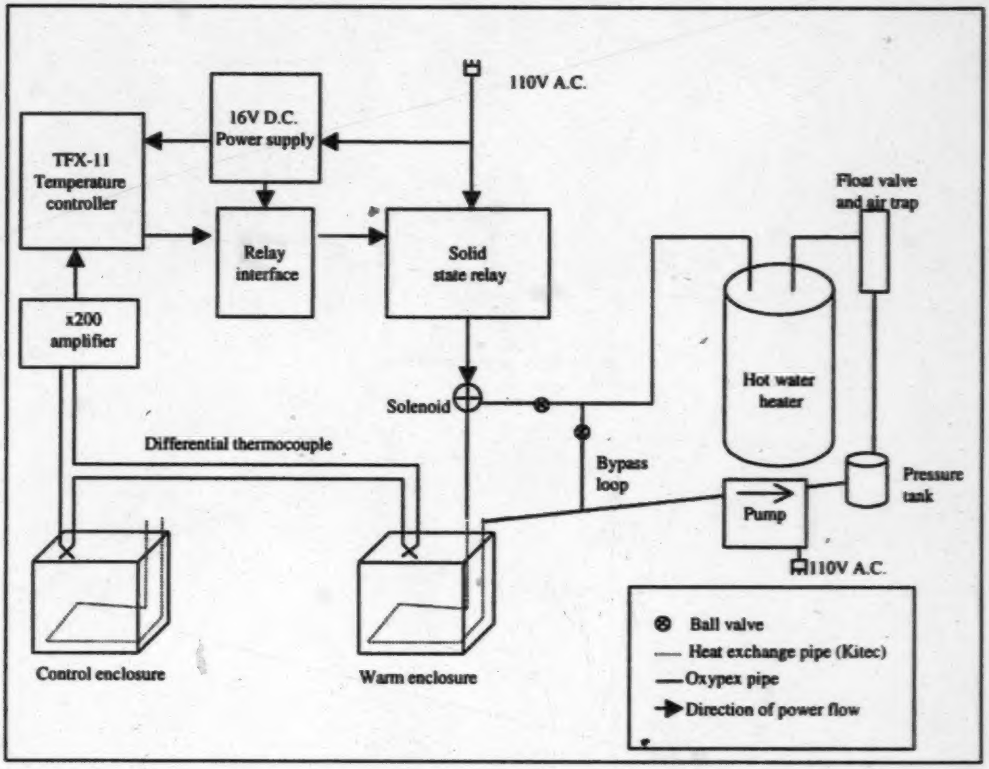
110V A.C.
16V D.C.
Power supply
TFX-11
Temperature
controller
Relay
interface
Solid
state relay
Float valve
and air trap
x200
amplifier
Hot water
heater
Solenoid
Differential thermocouple
Bypass
loop
Pressure
tank
Pump
110V A.C.
Control enclosure
Warm enclosure
Ball valve
Heat exchange pipe (Kitec)
Oxypex pipe
Direction of power flow

Figure 2.1: Simplified plumbing and wiring diagram. If the temperature difference between enclosures exceeded 5°C, the solenoid valve was closed and water flowed through the bypass loop. If the sensed temperature difference was less than 5°C, the solenoid valve was energized and water was allowed to flow through the heat exchange pipe in the warmed enclosure. Temperature differences were measured using a differential thermocouple, with a signal amplifier input into the temperature controller. The designed system included a second pair of enclosures heated using the same system with a second solenoid loop attached to the plumbing system and second differential thermocouple attached to the TFX-11 temperature controller.

valves when the sensed temperature difference fell below 5°C and closed them when this temperature difference exceeded 5°C. The temperature difference between paired enclosures was measured every second. However, this could be adapted by changing the software to allow increased or decreased frequency of monitoring.

The TFX-11 is subject to a degree of offset error. This error could be up to 0.5°C per channel, and the first input channel was particularly vulnerable to this error. The magnitude of offset was measured by applying a known voltage to the amplifier inputs using a voltage calibrator (1μV resolution) and noting the difference between the temperature reported by the controller, and the output signal (converted to temperature using standard tables). The offset for each input channel was then corrected using the program TFBasic (Onset).

A drawback of electronic temperature control is its susceptibility to lightning. A lightning strike near the enclosures on August 30, 2000 incapacitated the electronic temperature control systems. Temperature was controlled manually (via direct valve manipulation) until repairs could be made. Damage to the controllers was minimal, although controller input channels and resistors downstream of the controller were affected. More effective grounding might have prevented this problem, although effective grounding at this bedrock dominated site is difficult. Alternatively, thermocouples could be removed from the water if a storm is predicted, although this would lead to loss of temperature control.

Plumbing of heat exchange system

Hot water (approximately 45°C), generated by using propane hot water heaters (33 Can. gallon, 36 000 BTU/hour), was pumped through insulated Oxypex pipes to a 10-m coil of Kitec® heat exchange pipe (½"-diameter Al-Pex, Ipex Inc.) in the bottom of each heated enclosure. Kitec® is an aluminum-imbedded pipe, with interior and exterior polyethylene coating. It is rated for use in potable water systems. At the output side of the coil, Kitec® was linked back to Oxypex using standard crimp fittings, and water was recycled into the input side of the hot-water heater (Figure 2.1). Hot-water rated, continuous-duty circulating pumps (March model 809 HS series; Little Giant CMD 100-5B) were used. Maximum pump capacity was 6.5

gpm (30 L per min.), although flow rates were lower due to resistance within the pipes.

In this experiment, two enclosures were heated using each heat exchange system. Water could therefore follow one of three routes: through enclosure 1, enclosure 2, or through the bypass loop. If closed solenoid valves prevented water from entering the heat exchange pipe in the enclosures, water would travel through the bypass loop. This design minimized the frequency of pump start up and shut down and prevented unnecessary wear. The bypass line was considerably shorter than the enclosure routes and hence had lower resistance. The length of pipe leading to each enclosure also differed, again translating to varied resistance. To prevent the water from preferentially flowing through the bypass loop, ball valves were installed to increase resistance on the bypass line and to equalize flow through each of the enclosure lines. These valves also permitted manual temperature control when the electronic system was disabled.

We included an air trap at the highpoint of the heat exchange system where gases, released by initial heating of the water, could accumulate without creating a vapour lock. Above the air trap a float valve (also called a radiator valve) allowed automatic gas release. A pressure tank and a pressure gauge were used to maintain and monitor pressure within the system. The system ran effectively at pressures from 0 to 30 psi without pressure loss or airlocks (we did not test beyond this pressure). The system was filled with water from Lake 239 and pressurized using a jet pump. Periodic inspection of the inside of pipes showed minimal biofilm growth and flushing was not required.

The placement of heat exchange pipes on the bottom of the enclosures was expected to increase vertical mixing within the experimental enclosures; however, this was necessary to allow complete heating of the enclosures. Preliminary experiments (performed in the Lobstick River, Alberta) had shown that strong thermal stratification resulted when heat exchange pipes were located near the top, or middle of enclosure. This was of particular concern in this experiment, as the community of interest was benthic, and stratification would limit the amount of heating reaching the bottom of the enclosures.

System performance

Temperature control was good, and paralleled natural diurnal temperature fluctuations (Figure 2.2). Inferior temperature control was seen during the period following the lightning strike on August 30 before repairs were completed September 7. Temperatures within control enclosures were quite similar to temperatures within the lake (Figure 2.3). However, maximum and minimum temperatures within control enclosures were often lower within enclosures than within the lake. Maximum temperatures within control enclosures showed greater deviation from lake temperatures than minimum temperatures. This suggests that shading by enclosures was a major cause of the differences in temperature.

Rates of propane consumption varied with differences in wind and wave action. The large variation in rates of propane consumption meant that periodically a tank ran empty, and the problem remained undiscovered for up to eight hours. Temperature control could be improved by linking multiple propane tanks, using larger volume gauged tanks, or using a telemetry system with alarm settings.

Effects of the heat treatment on the benthic boundary layer

The benthic boundary layer restricts nutrient transport into benthic communities, and as a result, differences in boundary layer thickness can affect the nutrient status and photosynthetic rates of a community (Riber and Wetzel, 1987, Turner et al., 1991). To determine whether benthic boundary layer thickness was affected by the heat treatment or by the presence of enclosures, gypsum chips were deployed on the lake bottom inside and outside of the enclosures. Gypsum chips were incubated for approximately two hours at uniform depths. Boundary layer thickness was calculated from the rate of weight loss of the chips using the method of Turner et al. (1991). On September 16th and September 18th boundary layer thickness was measured in two warm enclosures, in two control enclosures and at two lake sites (north and south of the enclosures). On September 24th boundary layer thickness was measured at night in all four control enclosures and two warm

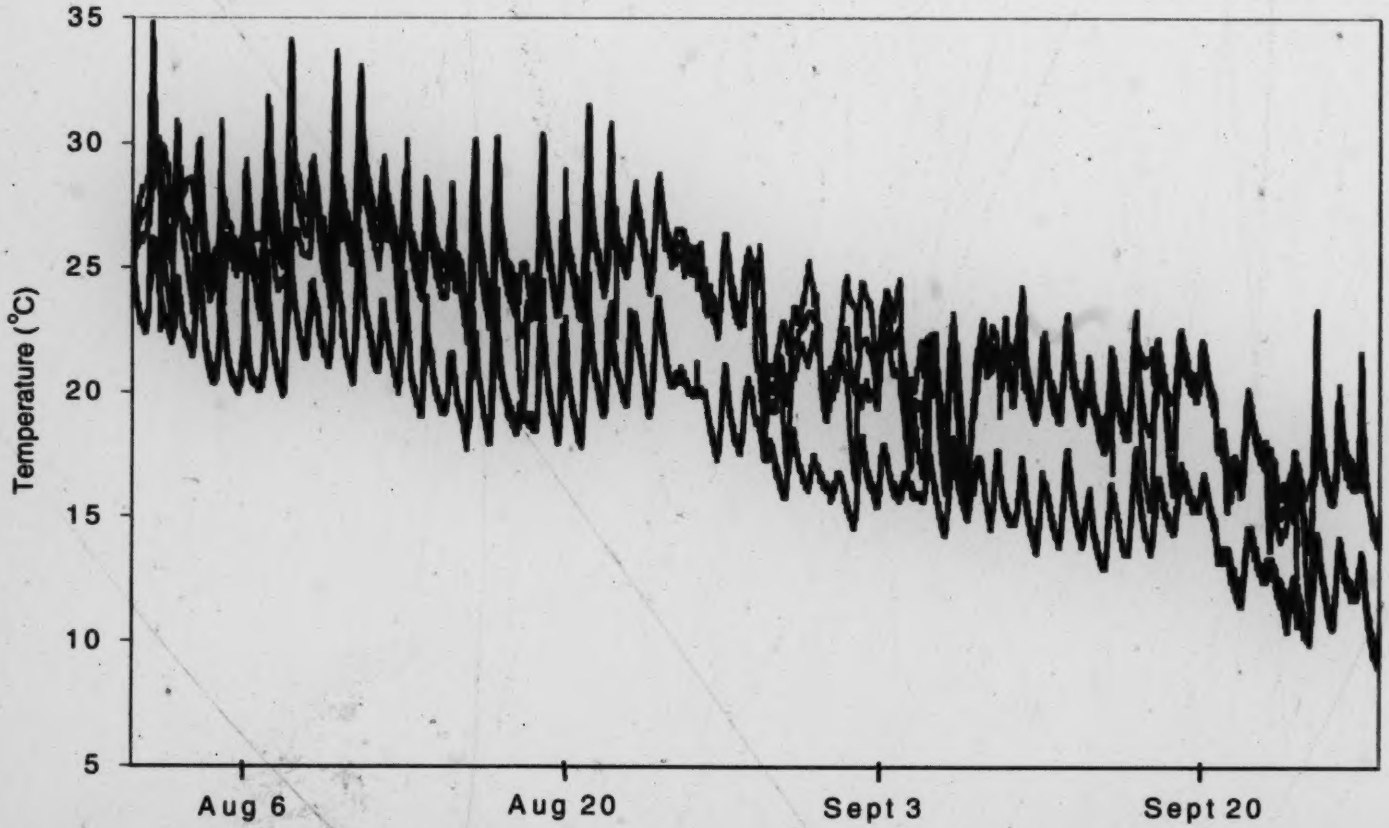

Figure 2.2: Temperature in warm and control enclosures. The mean temperature in warm enclosures is shown in black with red lines showing ±1 standard error. The mean temperature in control enclosures is shown in grey with blue lines showing ±1 standard error. Temperature was monitored every 15 minutes.

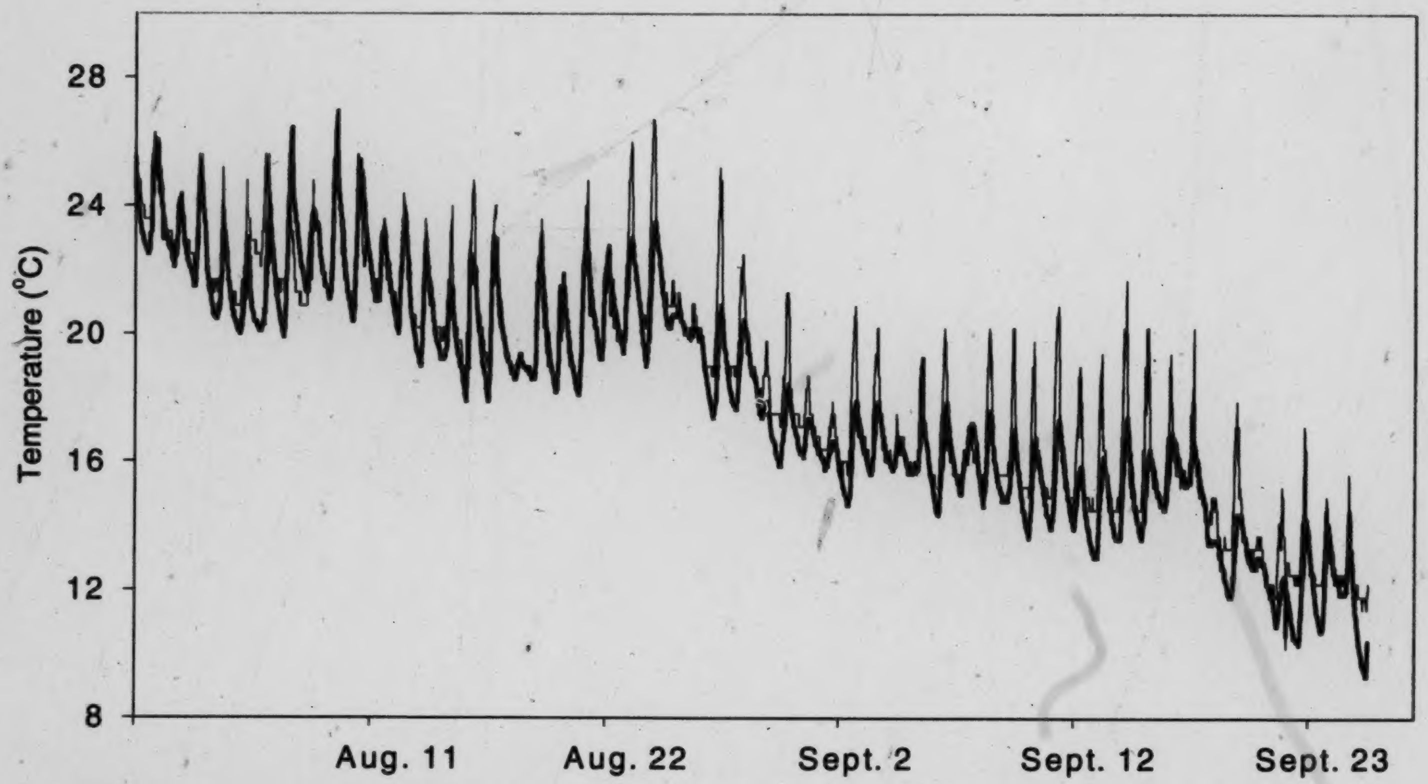

Figure 2.3: Mean temperature in control enclosures (blue line) and temperature in the lake adjacent to enclosures (black line). Temperature was measured every 15 minutes.

enclosures. Data from each sampling date were analyzed using one-way ANOVAs (SYSTAT 8.0). Data were log or power transformed (Taylor, 1961) as required to meet the assumptions of the analyses.

Boundary layer thickness was 2-4% greater in warm enclosures than control enclosures when measured during the day, and this difference increased to 18% at night (Figure 2.4). Differences in the daytime were not statistically significant (Table 2.1). Boundary layers in warm enclosures were significantly thicker than in controls at night. This difference is likely driven by increased solubility and rates of diffusion in warmer waters. Increased convection due to heating from the bottom does not appear to have affected benthic boundary layers. The thickness of the benthic boundary layer should be monitored in any application of this experimental design, and effects on the study communities should be considered. The 2-4% difference in daytime boundary layer thickness was expected to have minimal effects on the photosynthetic rates of experimental communities (Chapter 3).

Boundary layer thickness within enclosures was intermediate between values to the north and south of enclosures (Figure 2.4; Table 2.1). This indicates that the thickness of the benthic boundary layer was within the range of natural variability observed in the littoral zone; however, the range of this variability is broad.

Table 2.1: Results of 1-way ANOVAs on boundary layer thickness.

Date	Source of variation	df	F	p
Sept 16, 2000, day	Control, warm, outside enclosures	2	0.18	0.84
Sept 18, 2000, day	Control, warm, outside enclosures	2	0.21	0.82
Sept 24, 2000, night	Control vs. warm	1	15.6	0.02

Enclosure design

Enclosures were made of woven polyethylene with an external wooden frame. Enclosures were insulated with a translucent closed-cell foam packaging material (Shippers Supply Inc.) attached to the wooden frames. Three layers of 1/8" insulation were wrapped around the enclosures. All enclosures were 1.4 m x 1.4 m, with sloping bottoms that approximately paralleled the lake bottom. The average

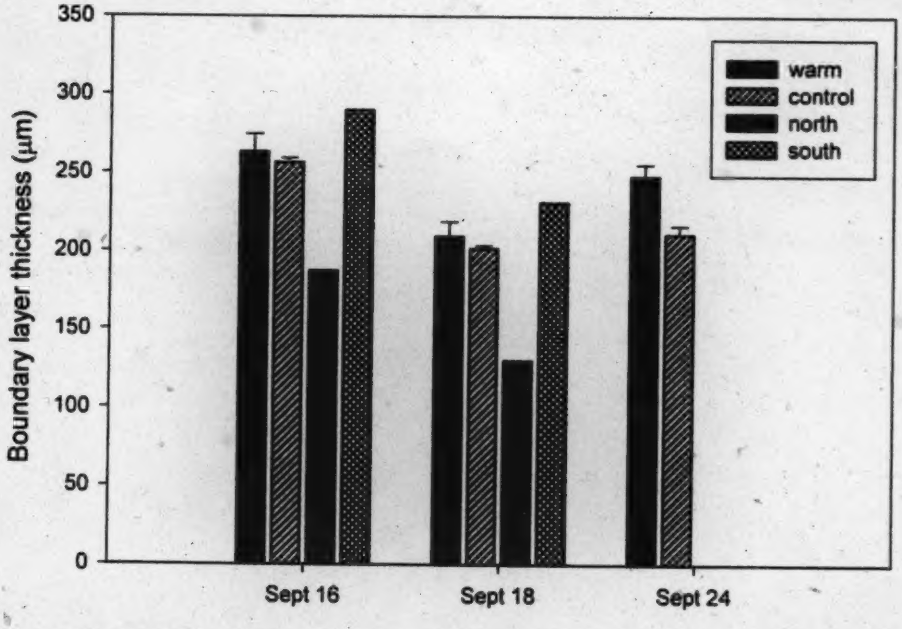

Figure 2.4: Boundary layer thickness outside enclosures, and in warm and control enclosures. On September 16th and September 18th boundary layer thickness was measured during the daytime in two warm enclosures, two control enclosures, one site north of the enclosures and one site south of the enclosures. On September 24th boundary layer thickness was measured at night in all four control enclosures and two warm enclosures. Error bars (where included) show ±1 standard error.

enclosure volume on September 27th was 700 L (estimated by NaCl addition), and the mean depth of the enclosures over the course of the experiment was 49 cm. Enclosures were open to the bottom, and secured by placing sandbags on a 0.7-m wide skirt. The sandbags did not completely seal the enclosures from the lake; instead, water exchange occurred. The mean water residence time in the enclosures was 5.6 days (estimated via NaCl addition, September 26-27, 2000). Although the fabric may not have totally prevented water exchange, we suspect that the bottom-seal was the major point of leakage. Warmed enclosures showed greater leakage rates (244 ± 118 [SD] L • d^{-1}) than control enclosures (166± 153 [SD] L • d^{-1}). However, differences in water chemistry between warmed and control enclosures were minor (Chapter 3).

Periodically strong waves tore the insulation away from the enclosures. Rapid heat loss then ensued. A more robust insulation is clearly desired. Wrapping the insulation with a thick plastic sheet might provide the required protection. The use of foam insulation increased the buoyancy of the enclosures, but placement of sandbags around the enclosure skirts and rocks on top of the wooden enclosure frames counteracted this buoyancy. Unfortunately, preliminary tests of other insulation materials, including water jackets and bubble wrap showed poor heat retention in water.

Propane consumption varied from 120 000 – 540 000 BTU per enclosure per day (mean=220 000 BTU per enclosure per day). Energy efficiency could be improved by reducing the rate of water exchange between the enclosures and the lake, either by improving the seal between the enclosure and substratum, or by using a closed-bottom enclosure. With more effective insulation, and reduced water exchange, a single propane hot water heater might warm four enclosures of this size. However, heating two enclosures with one hot water heater was the effective limit of our design, as evidenced by the mean temperature difference of 4.5°C during the experiment. During periods of high winds and waves, 5°C temperature differences were difficult to maintain. A boiler-driven system that adjusted hot water temperatures based on heat demand would further improve efficiency, but was considered too complicated for this field application.

Enclosure effects

We monitored carbon accrual on enclosure fabric and accrual of carbon and pigments on pipes to determine whether the enclosures, or the heating system would affect study communities. Strips of enclosure fabric were installed on August 8th and removed on September 25th. Fabric strips were measured for area, and the biofilm was scraped into a known volume of lake water. Samples were obtained from the Kitec® pipe on September 25th, 2000 after an eight-week incubation. Suspensions of the biofilm were blended for 10 seconds to homogenize the samples. Suspensions were then transferred to stirred beakers, and subsampled using a wide-bore syringe. Samples for carbon and nitrogen analyses were dried, then frozen and analyzed using a CHN Control Equipment Corporation 440 Elemental Analyzer. Samples for pigment analysis were processed as described in Chapter 3. Differences in carbon accrual between warm and control enclosures were assessed using randomized block (RB)- ANOVAs. Effects on pigment accrual were first probed using a randomized-block multivariate-ANOVA (RB-MANOVA), then with individual RB-ANOVAs. Data were transformed as necessary to meet the assumptions of the analyses. All tests were performed using SYSTAT 8.0.

Enclosure fabric and heat exchange pipes showed considerable carbon accumulation. Carbon accumulation on fabric did not differ between warm and control enclosures (Table 2.2). On average, more carbon accumulated on hot water pipes than on pipes within control enclosures, although this result was marginally non-significant (p=0.053, Table 2.2). Given that the pipe surface area was approximately 20% of the bedrock surface area, this increased biofilm accumulation could lead to accelerated nutrient depletion in warmed enclosures if water exchange had not occurred between enclosures and the lake.

Table 2.2: Carbon accumulation (μg cm^{-2}) on pipes and enclosure fabric. Standard deviations are indicated in brackets. RB-ANOVAs were performed, and statistical output is shown. One df was associated with the heat treatment and three df were associated with each block.

	Warm	Control	*F*	*p*
Pipe	273 (100)	106 (62)	9.67	0.053
Fabric	16 (8)	17 (18)	0.001	0.97

We expected that the algal community on hot water pipes would be dominated by high-temperature tolerant species such as Cyanobacteria and chlorophytes (DeNicola, 1996). In contrast, we expected that the algal community on pipes within control enclosures would be similar to the diatom-dominated tile communities (Chapter 4). Although pigment concentrations may be affected by a multitude of factors other than taxonomic change, including temperature (Rosen and Lowe, 1984; Deventer and Heckman, 1996; Schofield et al., 1998; Coles and Jones, 2000), we have used pigments as a rough approximation of algal taxonomy, with these limitations in mind. Given that increased temperatures can lead to changes in cellular pigment content, these results should be interpreted with considerable caution (Huner et al., 1998; Coles and Jones, 2000). Increased accrual of chlorophyll *a* and β-carotene may indicate that total algal biomass was greater on heated pipes. The increase in carbon accrual on heated pipes, although not statistically significant, is consistent with increased algal biomass. However, increases in the abundance of other community constituents including invertebrates, bacteria or detritus could also drive this change. Increased accrual of fucoxanthin (chrysophytes, diatoms, some dinoflagellates), canthaxanthin (Cyanobacteria), chlorophyll *b* (chlorophytes, euglenophytes) and violaxanthin (chlorophytes) on heated pipes may indicate that algal communities of heated and unheated pipes differed (Table 2.3).

Table 2.3: Effect of the heat treatment on pigment accrual on pipes. The multivariate test of heat treatment effects on the seven pigments is reported (RB-MANOVA) and univariate tests (RB-ANOVA) are shown for each pigment. 3 df are associated with the each block, and 1 df is associated with the heat treatment. Data shown are means (nmol•m^{-2}) with standard deviations indicated in brackets. Taxa associated with the presence of pigments are listed (Leavitt, 1993; Vinebrooke and Leavitt, 1999)

	Taxa	Control	Warm	*F*	*p*
MANOVA				51	0.01
Chlorophyll *a*	All algae	780 (570)	4510 (2960)	11	0.04
Chlorophyll *b*	Chlorophytes, euglenophytes	125 (75)	1230 (900)	62	0.004
β-carotene	All algae	130 (94)	735 (466)	160	0.001
Fucoxanthin	Diatoms, chrysophytes, dinoflagellates	798 (360)	3050 (1640)	18	0.02
Lutein/zeaxanthin	Chlorophytes, Cyanobacteria	194 (90)	1750 (870)	16	0.02
Canthaxanthin	Cyanobacteria	54 (23)	200 (58)	33	0.01
Violaxanthin	Chlorophytes	28 (20)	305 (187)	27	0.01

Differences in the abundance of carbon and pigments on warm and control pipes indicate that it is important that the community of interest is separated from the pipes. We ensured that our study communities were separated from the pipes by a minimum of 10 cm. In the future, studying the algal community at varying distances from the heating pipes would be advantageous to determine whether a 10-cm separation is sufficient to prevent study communities from being affected by communities on the heat exchange pipes.

Summary

This experimental apparatus was used successfully in an experiment (August-September 2000) designed to assess climate change effects on benthic community composition and metabolism (Chapter 3, Chapter 4). The design can be easily applied to other littoral sites or could be modified to allow study of climate change effects within the pelagic zone. The design could also be modified to allow study of altered stratification patterns on planktonic communities. Effects of the experimental apparatus on study communities should be considered, and biofilm development on enclosures and pipes should be monitored.

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Chapter 3: Effects of increased temperature on epilithic metabolism

Introduction

Current climate models predict that global surface temperatures will increase by 1.4 to 5.8°C between 1990 and 2100 (Intergovernmental Panel on Climate Change, 2001). Surface water temperatures of stratified lakes in the Precambrian Shield and Laurentian Great Lakes region are projected to increase 1-7°C over this same period (Magnuson et al., 1997). Although bottom-dwelling communities provide up to 50% of the energy required for fish production in lakes (Hecky and Hesslein, 1995), little is known about the effects of climate warming on these communities (DeNicola, 1996). In this study, we investigated how climatic warming may affect lakes by examining the metabolic responses of epilithon (the biofilm on rock surfaces composed of bacteria, fungi, invertebrates and detritus) to increased water temperatures. Lake metabolism and in particular epilithic metabolism, may be a sensitive indicator of ecosystem change (Schindler, 1990).

Relatively little is known about how temperature affects metabolism of epilithon. Studies of phytoplankton cultures have shown that temperature sets an upper limit for photosynthetic rates (Davison, 1991). Optimum temperatures for photosynthesis also vary among algal species, which suggests that taxonomic shifts caused by temperature increases could lead to increased photosynthetic rates (DeNicola, 1996). Hickman (1982) found that thermally polluted sites within Lake Wabamun had higher rates of primary production than sites unaffected by the thermal effluent. Phinney and McIntire (1965) also showed that photosynthetic rates were stimulated at elevated temperatures in laboratory streams. Increased photosynthetic rates could be associated with increased biomass. Seasonal changes in algal biovolume were positively correlated with temperature in the sediments of a British Columbia lake (Gruendling, 1971).

Respiration is also temperature dependent. Rates of epilithic respiration depend upon cumulative respiration rates of three major community constituents, algae, bacteria and invertebrates. All three groups exhibit increased respiration with increased temperature (Sweeney, 1978; Graham et al., 1996; Thamdrup and Fleischer, 1998; Höckelmann and Pusch, 2000; Pomeroy and Wiebe, 2001). Differences in the magnitude of temperature effects on rates of photosynthesis and respiration have driven changes in the proportion of fixed carbon allocated to respiration (Busch and Fisher, 1981), a stress response described by Odum (1985).

Although chlorophyll *a* is used as a biomass indicator in numerous aquatic studies, including studies that assess temperature influences on biomass (e.g. Hickman and Klarer, 1975), cellular concentrations of chlorophyll *a* and other pigments are temperature dependent in many species. These changes constitute a means by which algae may acclimate to different thermal regimes, and are not necessarily related to changes in algal biomass (Huner et al., 1998). In fact, cellular chlorophyll *a* concentrations increase in many algal taxa at elevated temperatures (Kübler and Davison, 1995; Coles and Jones, 2000). In addition, accessory pigments such as chlorophyll *b*, lutein and fucoxanthin often increase in parallel to chlorophyll *a*, although the photoprotective properties of carotenoids may complicate this relationship (Tang and Vincent, 1999).

In August-September 2000, we established eight littoral enclosures along the shoreline of Lake 239 (L239) at the Experimental Lakes Area (ELA) and experimentally warmed half of the enclosures by 4.5°C using a closed-circulation heat exchange system. We hypothesized that heating would stimulate photosynthetic rates by increasing rates of light-saturated photosynthesis. We expected to observe increases in ratios of chlorophyll *a* and accessory pigments to algal biomass. We hypothesized that respiration rates would increase, leading to an increase in the amount of fixed carbon allocated to respiration (R_{dark}:P_{gross}). The effect of temperature on rates of net photosynthesis, dark respiration and R_{dark}:P_{gross} was also studied using long-term epilithic data from L239. Further, we experimentally assessed whether changes in rates of photosynthesis and respiration were direct temperature effects, or reflected community change in the epilithon. Finally, we expected that increased rates of net photosynthesis would lead to increased epilithic biomass in warmed enclosures.

Materials and Methods

Long-term data

Using data collected in L239 at the Experimental Lakes Area (ELA) in northwestern Ontario (49°40'N, 93°44'W) from 1981 to 1997, we tested the hypothesis that rates of epilithic metabolism were correlated with water temperatures. We restricted our analysis to the months of August and September to allow comparison with experimental results. Metabolic incubations were performed in the middle littoral zone, at depths of approximately 1-2 m. In 1981 and 1982, incubations were performed in 0.85-L acrylic chambers that sealed to the substrata and enclosed 200 cm^2 of bedrock. Subsequent incubations were performed in smaller chambers (0.425 L) that enclosed 100 cm^2 of bedrock. Rates of net photosynthesis were measured in clear acrylic chambers by measuring dissolved inorganic carbon (DIC) uptake. Rates of dark respiration were measured in black acrylic chambers by measuring release of DIC (Turner et al., 1983). Typically, three replicate light and dark chambers were incubated, and the respective means were used in the analyses. Rates of gross photosynthesis were estimated by adding the absolute value of dark respiration rates to rates of net photosynthesis, allowing the proportion of fixed carbon allocated to respiration (R_{dark}:P_{gross}) to be estimated.

Data were analyzed using Model-I (least-squares) linear regression. Although some measurement error was expected in the predictor variable (temperature), the magnitude of this error was expected to be very small relative to error in the response variable. Therefore model-I regression was suitable (McArdle, 1988). Data were inspected for normality, homogeneity of variances and linearity. The homogeneity of variances assumption was not met for dark respiration, so a transformation was identified using Taylor's (1961) power law, and data were square-root transformed. We tested for serial correlation of residuals using the Durbin-Watson statistic. All analyses were performed in SYSTAT 8.0.

Enclosures

Eight experimental enclosures were constructed on the western shore of L239. L239 is a 56-hectare oligotrophic lake with a maximum depth of 30.4 m. The shoreline has a gradual slope. Two study sites located on both sides of a small bedrock outcropping were selected. Four enclosures were installed at each site at approximately equal depths on the bedrock substratum. Each site was divided into two blocks to minimize differences in slope, substratum composition and light. Warm or control enclosures were randomly assigned within each block. Two of the enclosures at each site were experimentally warmed and two were maintained as controls. Water temperatures within control enclosures were similar to lake temperatures outside enclosures (Chapter 2).

Enclosures were made of woven polyethylene (Canfab products Ltd.) with an external wooden frame. All enclosures were 1.4 m x 1.4 m, with sloping bottoms that approximately paralleled the bedrock slope. Enclosures were open to the bottom and secured by placing sandbags on a 0.7-m wide skirt. The average enclosure volume at the end of the experiment was 700 L (estimated by NaCl addition) and the mean depth of the enclosures over the course of the experiment was 49 cm. Water exchange between the lake and enclosures was estimated at 5.6 days by NaCl addition at the end of the experiment.

Half of the enclosures were experimentally warmed by approximately 4.5°C above control temperatures using a heat exchange system. The heat treatment was established on August 3, 2000 and continued until September 27, 2000 for a total of 55 days. Water temperature was recorded every 15 minutes in each enclosure and in the lake using continuously recording thermocouples (HoboTemp, Onset).

The heat treatment was established by pumping hot water through a system of heat exchange pipes (Kitex Al-pex, Ipex Inc.) located in the bottom of the enclosures (pumps=March model 809 HS series; Little Giant CMD 100-5B). No water exchange occurred between the heating system and enclosures. Heat exchange pipes were also placed in control enclosures, but were not connected to the heating system (see Chapter 2 for more detail).

Warmed enclosures were paired with the nearest control enclosure and differential thermocouples were placed in enclosure pairs to detect the temperature difference. Thermocouples were attached to a TFX-11 controller (Onset) and the controller was programmed to produce a signal when the sensed temperature difference was less than 5°C. This signal was converted to a voltage that opened a solenoid valve (Asco electric) and allowed hot water to flow into the 10 m of ½-inch (1.3 cm) diameter heat exchange pipe (Kitec Al-Pex, Ipex Inc.) in the warmed enclosures. When the temperature difference exceeded 5°C, the controller stopped producing an output signal and the solenoid valve was closed. One heating system was used for both warmed enclosures at each study site. When temperature differences in both pairs of enclosures at a study site exceeded 5°C, water was shunted through a bypass loop (insulated Oxypex pipe). Resistance in the bypass loop and in each heating loop was regulated using globe valves to ensure appropriate distribution of hot water.

Artificial substrata

Unglazed ceramic tiles (4.8 cm x 4.8 cm) were combusted at 600°C for a minimum of one hour to remove organic residue. Tiles were scrubbed, soaked in dilute hydrochloric acid overnight, rinsed with deionized water and dried. Tiles were allowed to colonize for approximately eight weeks in L239 at a depth of 40-60 cm before being transferred to the enclosures where they were kept at least 10-cm away from the heat exchange pipes. Tiles were sampled at random using a matrix of random numbers corresponding to the tile distribution. Sampling was often split by blocks over several days, as shown in Table 3.1.

Table 3.1: Dates of incubations to measure rates of net photosynthesis and community dark respiration, and dates of sampling for carbon, pigments, and algal biomass.

	Net photosynthesis	Community dark respiration	Carbon, pigments, algal biomass
Blocks 1,2			August 5
Blocks 3,4			August 5
Blocks 1,2	August 13	August 14	August 14
Blocks 3,4	August 16	August 17	August 17
Blocks 1,2	August 22	August 25	August 25
Blocks 3,4	August 23	August 26	August 26
Blocks 1,2	September 13	September 11	September 13
Blocks 3,4	September 13	September 11	September 13
Blocks 1,2	September 21	September 22	September 22
Blocks 3,4	September 21	September 22	September 22

Epilithic metabolism

Rates of net primary production and community dark respiration were measured *in situ* by incubating eight tiles in 0.41-L (23 x 11.5 x 2.5 cm with 23 x 11.5x 0.6-cm inserts) plexiglass chambers and monitoring changes in DIC concentrations during the course of an approximately 90 minute incubation (Turner et al., 1983). Photosynthetic chambers were constructed of OP4 and chambers for measurement of dark respiration were made with black plexiglass. DIC concentrations were measured using an infrared gas analyzer. The same tiles were used for measurement of net primary production, dark respiration, algal biomass and particulate concentrations. The proportion of gross photosynthesis (estimated as the sum of the absolute value of dark respiration and net photosynthesis) allocated to respiratory carbon loss (R_{dark}:P_{gross}) was estimated using measurements of dark respiration and net photosynthesis on separate dates. Effects of warming on net photosynthesis, respiration and R_{dark}:P_{gross} were analysed using a repeated-measures (RM), randomized-block (RB) MANOVA and individual RM-RB-ANOVAs. A significance level of α=0.05 was selected for all analyses. For all ANOVAs and MANOVAs, data were inspected for adherence to the assumptions of the analysis. If the normality assumption was violated, data were log transformed. If the homogeneity of variances assumption was violated, data were transformed using Taylor's power law (1961).

To determine whether the heat treatment affected the maximum photosynthetic rate or the initial slope of the photosynthesis irradiance curve, three different light treatments were established by covering the clear acrylic chambers with black screens. Light levels within the chambers were approximately 584, 193 and 72 μE m^{-2} s^{-1} (Li190SA Li-Cor quantum cosine sensor). At each site composite warm and control samples were assembled using four tiles from each enclosure. Metabolic rates were measured as described previously at each light treatment and in dark chambers. Incubations of composite samples were performed in one warmed and one control enclosure at each site on August 30, 2000. The maximum photosynthetic rate and the initial slope of the photosynthesis irradiance curve were estimated using the Fee model (1998). Results were analyzed using one-way, fixed effects ANOVAs.

Several exchange experiments were performed to assess whether changes in metabolic rates were a direct metabolic effect resulting from increased temperature, or whether changes resulted from a shift in community composition. Tiles were exchanged between paired enclosures, allowed to acclimate for approximately one hour, and metabolic rates were measured. Data were first analyzed using RM-RB-MANOVAs, then with individual RM-RB-ANOVAs.

To allow comparison between metabolic rates of the natural bedrock community and tile community, net photosynthesis incubations on tiles within enclosures were run concurrently with incubations of epilithon on natural bedrock on Sept. 13, 2000. Static acrylic chambers were sealed to the rock surface and the change in DIC concentrations was measured over time (Turner et al., 1991). Two replicate net photosynthesis and dark respiration incubations were made on the rock substrata at each experimental site. Dark respiration rates measured on tiles two days earlier were compared with the rock measurements. Bedrock incubations were in slightly deeper water (0.49- 0.62m) than tile incubations (0.28-0.46m). One sample *t*-

tests were used to determine whether metabolic rates differed between tiles within control enclosures and the natural bedrock substrata within the lake.

Analyses of pigments, carbon and algal biomass

Following completion of metabolic measurements, the chambers containing tiles were removed and refrigerated for a maximum of 30 h. The biofilm on eight tiles from each metabolic chamber was scraped into 1 L of lake water using a plastic ruler. This composite suspension was blended for 10 s at low speed to homogenize the sample, transferred to a beaker and stirred. Subsamples for study of algal biomass were taken using a wide-bore syringe and preserved with acid Lugol's (4% final concentration). Algal counts were performed using the modified Utermöhl technique (Nauwerck, 1963) and a phase-contrast inverted microscope at 125x and 400x magnification. Measurements of algal cell biovolume were used to estimate algal wet biomass using regressions for different taxa (Vollenweider, 1974). Results were analysed using a RM-RB-ANOVA.

For analysis of algal pigments, subsamples of the slurry (5-10 mL) were filtered onto GF/C filters and frozen. They were later freeze-dried for 24 h at 100 millitor with the specimen chamber at 0°C and the condenser chamber at –40°C (Virtis Model 24DX49 Specimen Freeze Dryer). Dried samples were extracted in a solution of 80% acetone, 15% methanol and 5% water (by volume) for 24 h at 10°C in the dark. Extracts were filtered through a 0.2-μm membrane filter and the filtrate was dried under nitrogen gas until analysis. Dried extracts were dissolved in a known volume of injection solvent (70% acetone, 25% ion-pairing reagent, 5% methanol by volume) containing 3.2 mg L^{-1} of the internal standard Sudan II. Pigments were separated on a HPLC Model 1100 with an inline diode array detector, fluorescence detector and a 10-cm Varian Microsorb C18 column with 100-angstrom beads. Two duplicate suspensions from each enclosure were analysed and the mean of these two samples was used in statistical analyses.

Subsamples for carbon analyses (5-10 mL) were filtered onto pre-ashed GF/C filters, dried and frozen. Carbon analyses were performed using a CHN Control

Equipment Corporation 440 Elemental Analyzer. The mean of two duplicate subsamples from each enclosure on each date was used in statistical analyses.

Ratios of chlorophyll *a* and total carotenoids to carbon were studied on five dates and ratios of these pigments to algal biomass were studied three times during the experiment. Abundance of chlorophyll *a* and total carotenoids can be used as indicators of total algal biomass (Vinebrooke and Leavitt, 1998; Leavitt, 1993). The data were first analysed using RM-RB-MANOVAs. Then results for individual ratios were probed using RM-RB-ANOVAs. Ratios of pheophytin and total carotenoids to chlorophyll *a* were first analyzed using a RM-RB-MANOVA, then with individual RM-RB-ANOVAs. If treatment and time × treatment effects within MANOVAs were not statistically significant, these effects within individual tests were compared to a more conservative Dunn-Šidák adjusted *p*-value (Johnson, 1998).

Water chemistry

To determine whether enclosure water chemistry diverged from that of the lake, we sampled enclosures approximately bi-weekly. A single water sample was obtained from just below the water surface of each enclosure and analysed according to Stainton et al. (1977). Results were compared to integrated epilimnetic water samples or samples obtained from a depth of 1m at the deepest part of the lake. These lake water samples were collected as a part of the long-term monitoring program at the ELA.

Results

Long-term data analysis

Net photosynthesis, dark respiration and R_{dark}:P_{gross} were all significantly and positively correlated with water temperature. Temperature explained 17% to 34% of the variance in these parameters (Figure 3.1).

Experimental results

Metabolism

Rates of net photosynthesis increased by 28-50% as a result of the heat treatment, and varied significantly over time (Figure 3.2, Table 3.2). Dark respiration rates in warmed enclosures were 29-51% higher than controls and also differed over time (Figure 3.2, Table 3.2). There was a statistically significant time × treatment interaction effect on the proportion of gross photosynthesis (estimated as the sum of net photosynthesis and the absolute value of dark respiration) allocated to respiratory carbon loss (Table 3.2). This effect appears to be driven by higher R_{dark}:P_{gross} in warmed enclosures on the final two sampling dates (Figure 3.2), after ambient water temperatures had begun to decline (Chapter 2).

The natural bedrock community had lower rates of net photosynthesis than the tile community (p=0.01, Table 3.3). Respiration rates were also lower on the bedrock substratum, although differences were marginally non-significant (p=0.051, Table 3.3). The ratio of dark respiration to gross photosynthesis was higher on tiles than on bedrock, but results were not statistically significant (p=0.08, Table 3.3).

In control enclosures the source of the tiles had no significant effect on photosynthetic rates. However, in warm enclosures the warm-enclosure communities had significantly higher rates of net photosynthesis than control-enclosure communities (Figure 3.3, Table 3.4). There was no statistically significant difference between rates of dark respiration on tiles colonized within warm enclosures and tiles transferred to warmed enclosures (Figure 3.3, Table 3.4). There was a significant time × treatment

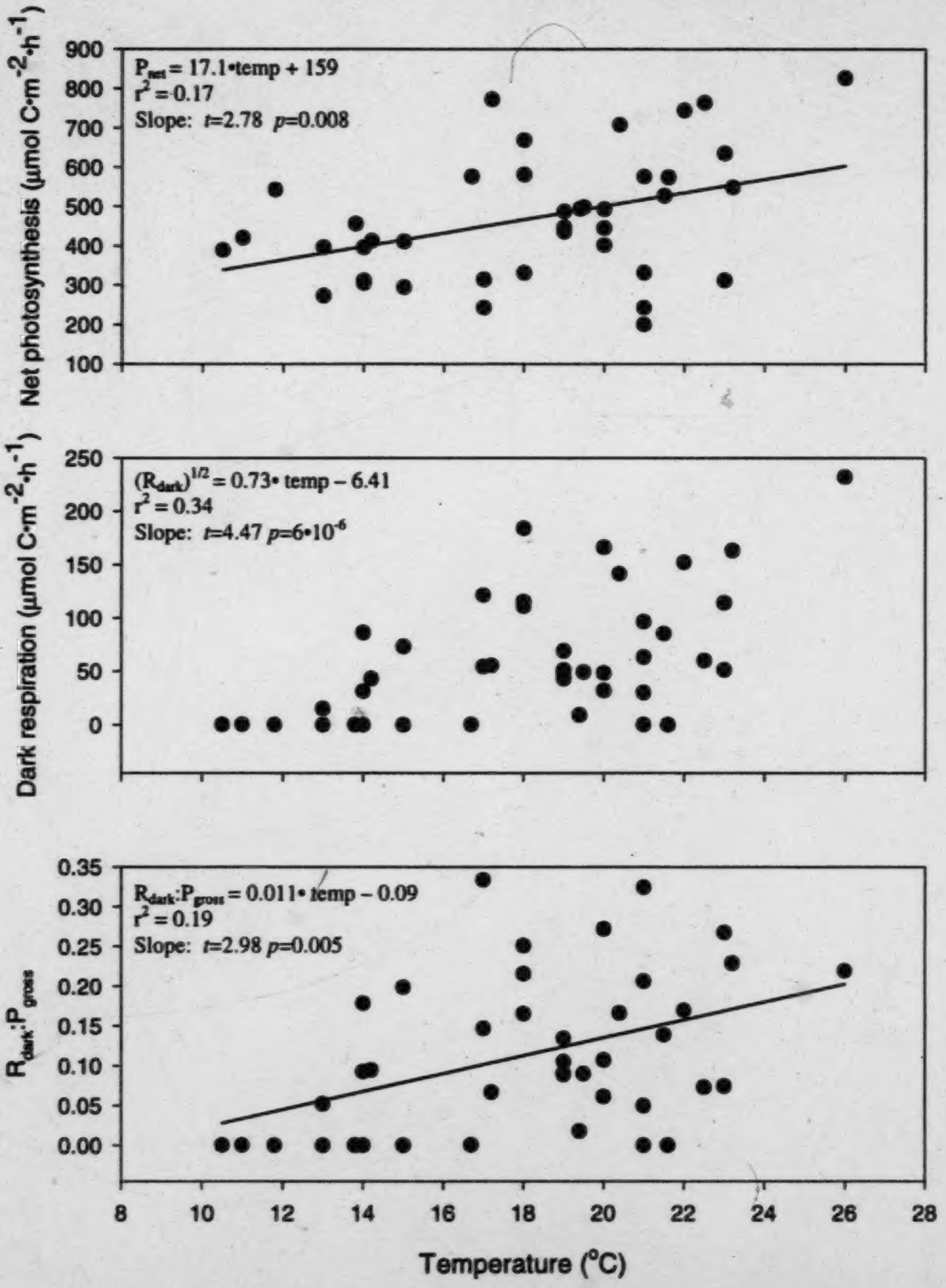

Figure 3.1: Relationship between temperature and metabolic variables in August-September, 1981-1997. The equations of the regression lines, r^2 values, and output of significance tests on the slope of the regression line are listed. No regression line is shown for R_{dark} because data were square root transformed prior to analysis. However the equation for the regression line is listed. 41 data points were included in each analysis.

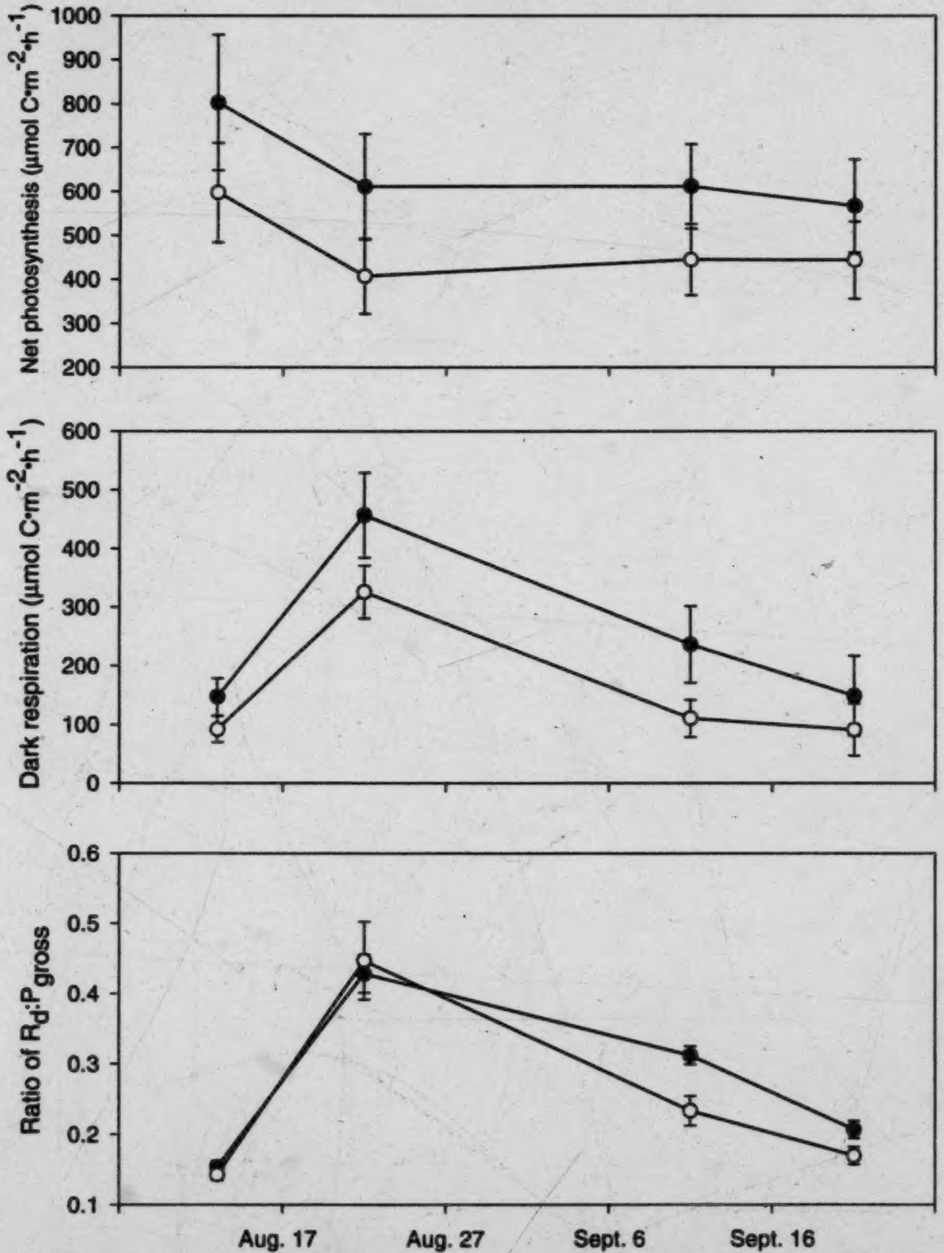

Figure 3.2: Rates of net photosynthesis, dark respiration and the ratio of dark respiration to gross photosynthesis, in control (white circles) and warm (black circles) enclosures. Error bars show ±1 standard error.

Table 3.2: Results a RM-RB-MANOVA and univariate tests on the effect of the heat treatment on rates of net photosynthesis, community dark respiration and the ratio of dark respiration to gross photosynthesis during the experiment.

Source of variation	df	*F*	*p*
MANOVA			
Heat treatment	1	62.1	0.004
Time	3	$1.5 \cdot 10^{4}$	$1.0 \cdot 10^{-15}$
Time × treatment	3	8.8	0.005
Net photosynthesis			
Heat treatment	1	28.7	0.013
Time	3	22.0	0.0002
Time × treatment	3	2.4	0.13
Dark respiration			
Heat treatment	1	65.5	0.004
Time	3	41.8	$1.3 \cdot 10^{-5}$
Time × treatment	3	0.6	0.56
$R_{dark}:P_{gross}$			
Heat treatment	1	50.1	0.006
Time	3	193	$1.7 \cdot 10^{-8}$
Time × treatment	3	4.70	0.031

Table 3.3: Metabolic rates on tiles within control enclosures and on the natural bedrock substrata outside enclosures. Data shown are means. Standard deviations are indicated in brackets. One-sample *t*-tests assessing the hypothesis that measurements within control enclosures are equal to the mean measurement on the bedrock substratum were performed, and the statistical output is shown. Three df were associated with each analysis.

	Tiles	Bedrock substratum	*t*	*p*
Photosynthesis	444 (20)	371 (55)	7.44	0.01
Respiration	137 (36)	80 (29)	3.14	0.051
$R_{dark}:P_{gross}$	0.23 (0.04)	0.18 (0.07)	2.55	0.08

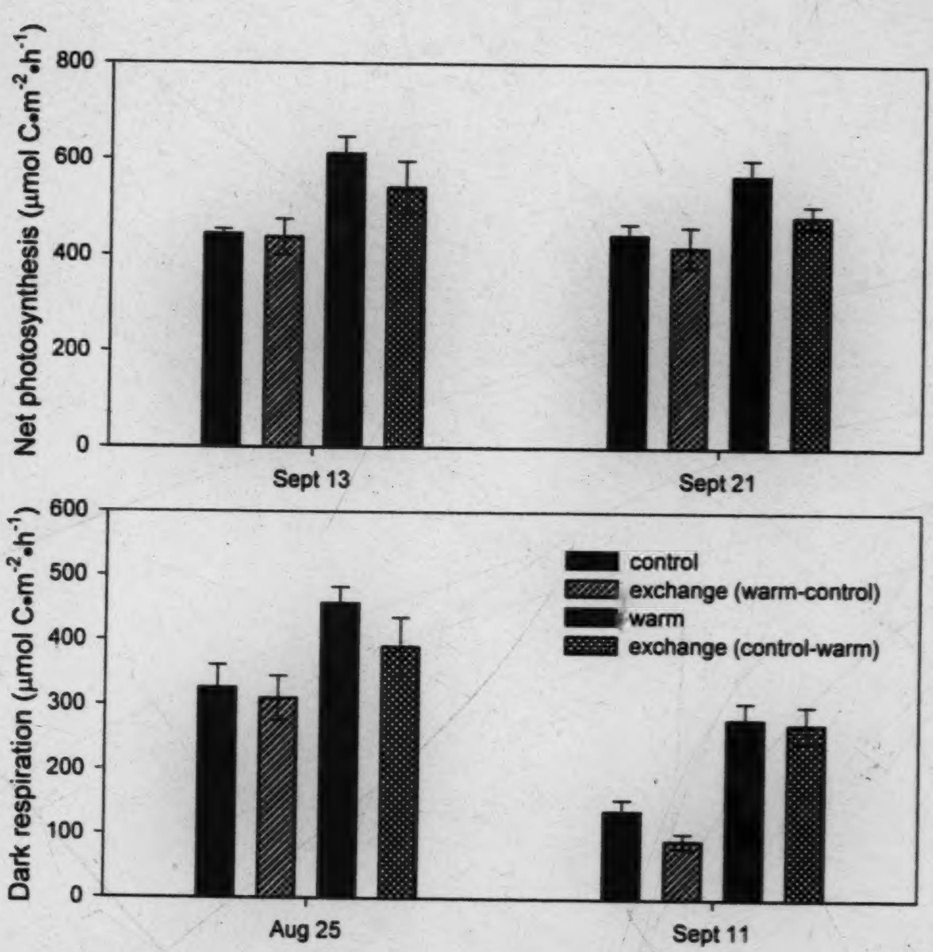

Figure 3.3: Rates of net photosynthesis and dark respiration in control and warm enclosures on tiles maintained within, and transferred between treatments. The direction of exchange is indicated in brackets, with the tile source listed first, and the tile destination listed second. Error bars show ±1 standard error.

Table 3.4: Effect of colonization history on rates of net photosynthesis and dark respiration during exchange experiments. RM-RB-MANOVAs and RM-RB-ANOVAs were used to test the hypotheses that rates of net photosynthesis or dark respiration differed between tiles that were maintained within an enclosure, and those transferred to an enclosure from the differing heat treatment. Results of statistical analyses are shown. The direction of exchange is indicated in brackets, with the tile source listed first, and the tile destination listed second.

Date	Source of variation	df	*F*	*p*
Net photosynthesis				
MANOVA	Heat treatment	1	20.9	0.02
	Time	1	29.0	0.01
	Time × treatment	1	2.04	0.25
Control vs. exchange (warm to control)	Heat treatment	1	0.37	0.59
	Time	1	0.75	0.45
	Time × treatment	1	0.63	0.49
Warm vs. exchange (control to warm)	Heat treatment	1	13.6	0.03
	Time	1	1.84	0.27
	Time × treatment	1	0.04	0.85
Dark respiration				
MANOVA	Heat treatment	1	102	0.002
	Time	1	7.21	0.07
	Time × treatment	1	0.05	0.84
Control vs. exchange (warm to control)	Heat treatment	1	9.12	0.06
	Time	1	719	0.0001
	Time × treatment	1	20.4	0.02
Warm vs. exchange (control to warm)	Heat treatment	1	3.88	0.14
	Time	1	33.1	0.01
	Time × treatment	1	1.34	0.33

interaction effect on respiration rates of tiles transferred from warm enclosures to controls, and those maintained within control enclosures (Figure 3.3, Table 3.4). Increases in respiration rates in warmed enclosures appear to be more strongly related to incubation temperature than colonization history.

The maximum photosynthetic rate was stimulated by the heat treatment; however, no effect of temperature on light-limited photosynthesis was observed (Figure 3.4, Table 3.5).

Pigments

Although ratios of chlorophyll *a* and carotenoids to algal biomass showed no statistically significant differences between warm and control enclosures (Table 3.6), mean pigment accrual was consistently greater in warmed enclosures (Chapter 4). Ratios of these pigments to carbon were significantly greater in warmed enclosures (Figure 3.5, Table 3.6). Ratios of pheophytin and carotenoids to chlorophyll *a* were typically higher in warmed enclosures, but these differences were not statistically significant (Figure 3.6, Table 3.7).

Biomass

Despite changes in rates of net photosynthesis, there was no effect of the heat treatment on algal biomass (Figure 3.7). Algal biomass was affected by an interaction between time and treatment effects (Table 3.8). This interaction appears to be driven by differences in biomass observed shortly after the initiation of the experiment that disappeared over time (Figure 3.7).

Water chemistry

Water chemistry was generally similar within warm and control enclosures. However, average concentrations of suspended phosphorus and nitrate were often greater in control enclosures than warmed enclosures. Transient differences in chlorophyll *a* concentrations and suspended nitrogen concentrations were also noted (Table 3.9).

Enclosure water chemistry was similar to epilimnetic water chemistry in L239 (Table 3.9), nevertheless some differences were noted. Average concentrations of ammonium and suspended nitrogen were often higher within the lake, and concentrations of total dissolved nitrogen were generally higher within enclosures. Nitrate concentrations were higher within the enclosures for the first half of the experiment and lower within enclosures during the second half. Concentrations of DIC were often slightly higher within enclosures. Total dissolved phosphorus (TDP) concentrations were similar within the lake and in the enclosures except in early September when concentrations within the lake were below detection limits. Concentrations of chlorophyll *a* and suspended carbon were often greater within the lake than in enclosures. This difference is quite marked in late September when a phytoplankton bloom was occurring with the lake and concentrations of chlorophyll *a* and suspended carbon were unusually high.

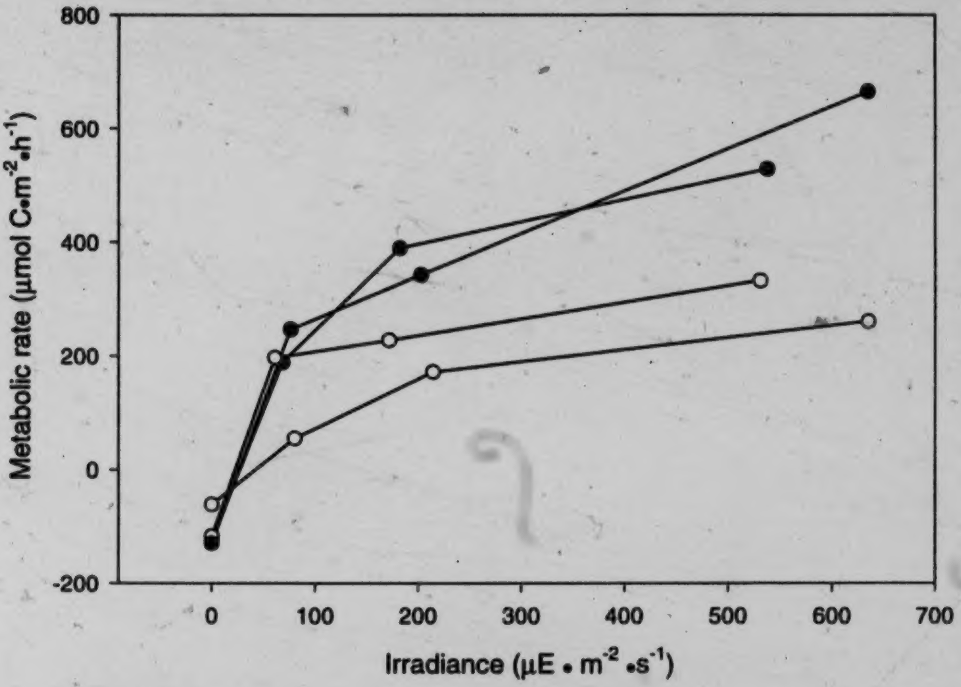

Figure 3.4: Net photosynthesis-irradiance relationships in control (white circle) and warm (black circle) enclosures on August 30, 2000. Negative metabolic rates show rates of dark respiration.

Table 3.5: Maximum photosynthetic rate (P_{max}, µM C • m^{-2} • h^{-1}), and the initial slope of the photosynthesis-irradiance curve (α, µM C • E^{-1}) on August 30, 2000 in warm and control enclosures. Data shown are means from two enclosures (± standard deviation), and the results of one-way fixed-effects ANOVAs assessing effects of the heat treatment on these parameters.

Variable	Control	Warm	*F*	*p*
P_{max}	265 (16)	571 (95)	58.84	0.02
α	1230 (1125)	1175 (131)	0.08	0.80
n	2	2		

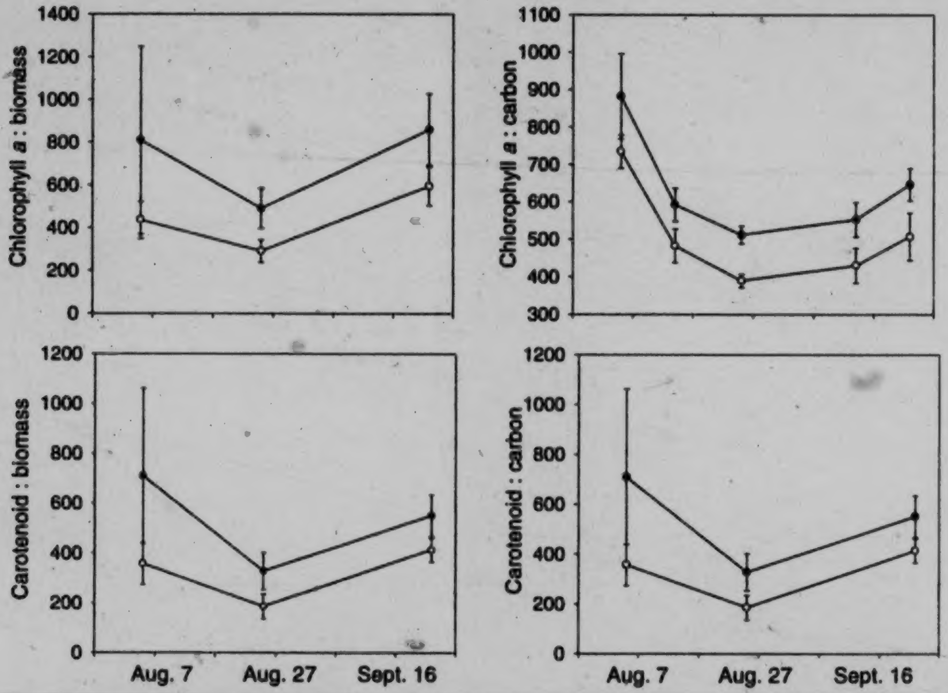

Figure 3.5: Algal pigment content (μg pigment • g algae^{-1}) and pigment concentrations normalized to carbon (μg pigment • g carbon^{-1}) in warm (black circles) and control (white circles) enclosures. Error bars show ±1 standard error.

Table 3.6: Results of RM-RB-ANOVAs on the effect of the heat treatment on the ratios of various pigments to algal biomass and to carbon. The multivariate tests incorporating results for the ratios of the four pigment groups to carbon and to biomass are reported (RM-RB-MANOVA). Because the MANOVA on pigment to biomass ratios was non-significant for treatment and time × treatment effects, these effects within the univariate tests should be compared to the more conservative Dunn-Šidák adjusted *p*-value of 0.025 (α=0.05, number of groups=2).

Source of Variation	df	*F*	*p*
MANOVA pigment: biomass			
Heat treatment	1	3.69	0.15
Time	2	12.4	0.007
Time × treatment	2	0.04	0.96
MANOVA pigment: carbon			
Heat treatment	1	59.2	0.005
Time	4	90.3	$7.5 \bullet 10^{-9}$
Time × treatment	4	1.13	0.39
Chlorophyll a*: biomass*			
Heat treatment	1	3.01	0.18
Time	2	5.00	0.052
Time × treatment	2	0.72	0.52
Chlorophyll a*: carbon*			
Heat treatment	1	17.0	0.03
Time	4	25.6	$1 \bullet 10^{-5}$
Time × treatment	4	0.08	0.99
Carotenoids to biomass			
Heat treatment	1	3.58	0.15
Time	2	5.85	0.04
Time × treatment	2	0.40	0.69
Carotenoids to carbon			
Heat treatment	1	131	0.001
Time	4	57.4	$1 \bullet 10^{-7}$
Time × treatment	4	2.00	0.16

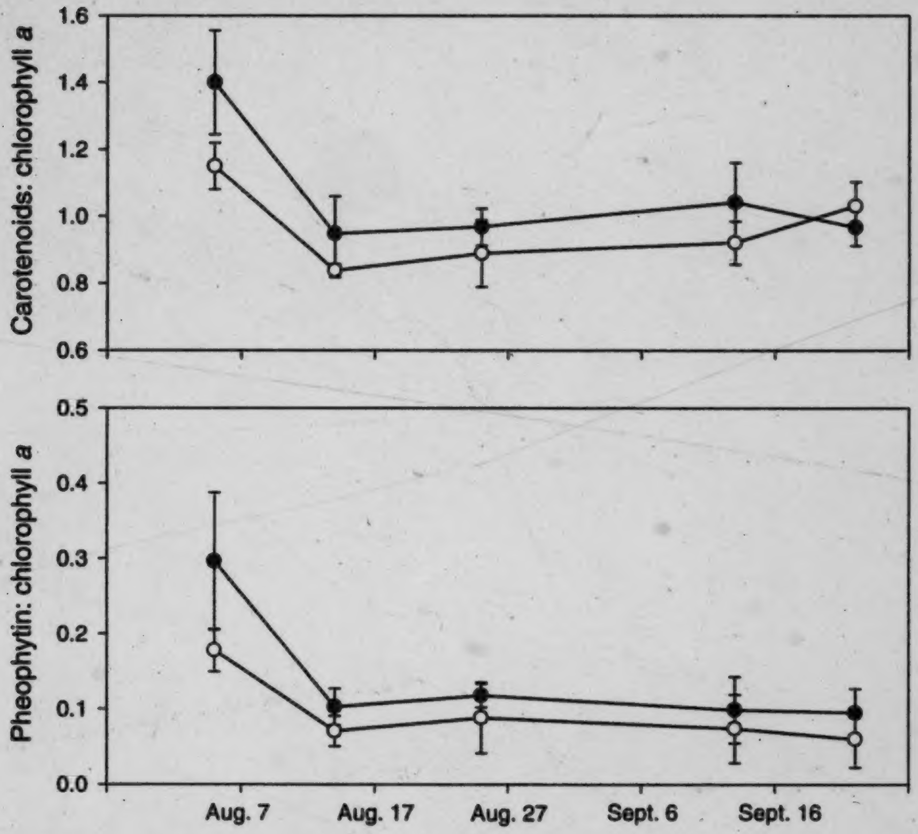

Figure 3.6: Molar ratios of pigments to chlorophyll *a* in warm (black circles) and control (white circles) enclosures. Error bars show ±1 standard error.

Table 3.7: Results of RM-RB-ANOVAs on the effect of the heat treatment on ratios of pheophytin and carotenoids to chlorophyll *a*. Because treatment and time × treatment effects in the overall MANOVA were non-significant, these results in the univariate tests should be compared to the Dunn-Šidák adjusted *p*-value of 0.013 (α=0.05, number of groups=2).

Source of Variation	df	*F*	*p*
MANOVA			
Heat treatment	1	3.12	0.18
Time	4	206	$6 \cdot 10^{-11}$
Time × treatment	4	0.53	0.72
Pheophytin: Chlorophyll a			
Heat treatment	1	4.80	0.12
Time	4	5.44	0.09
Time × treatment	4	0.47	0.76
Carotenoids: Chlorophyll a			
Heat treatment	1	1.99	0.25
Time	4	5.01	0.01
Time × treatment	4	0.55	0.61

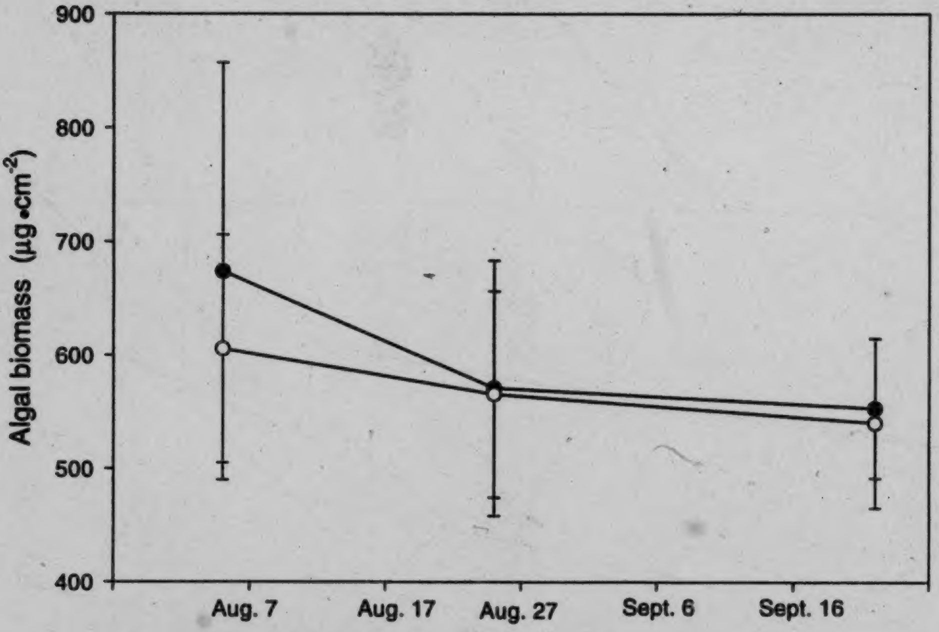

Figure 3.7: Algal biomass in control (white circles) and warm (black circles) enclosures. Error bars show ±1 standard error.

Table 3.8: Results of a RM-RB-ANOVA testing the effects of the heat treatment on algal biomass.

Source of Variation	df	*F*	*p*
Heat treatment	1	0.96	0.40
Time	2	2.18	0.19
Time × treatment	2	11.10	0.01

Table 3.9: Water chemistry in enclosures and in L239. Data reported are means ± SD of four samples. (If values were below the detection limit, a concentration of one half of the detection limit was assumed. If the chemical was not detected in any of the samples, the abbreviation n.d. is indicated. The presence of a dash indicates there are no results.). "Epi" samples are depth- integrated epilimnion samples.

		Aug. 9		Aug. 8	Aug. 23		Aug. 21	Sept. 7		Sept. 5	Sept 20		Sept. 18
	units	control	warm	Lake (epi)	control	warm	Lake (1m)	control	warm	Lake (epi)	control	warm	Lake (1m)
NO_3	$\mu g \cdot L^{-1}$	3.5 (0.6)	2.8 (1.0)	1	3.3 (3.9)	1.4 (1.1)	n.d	n.d.	n.d.	2	1.5 (0.6)	1.5 (0.6)	9
NH_4	$\mu g \cdot L^{-1}$	20 (4)	23 (11)	22	15 (3)	13 (4)	13	18 (3)	21 (4)	25	15 (2)	15 (4)	19
Susp. N	$\mu g \cdot L^{-1}$	56 (14)	53 (4)	62	52 (11)	63 (10)	75	70 (24)	61 (11)	78	72 (21)	50 (4)	149
TDN	$\mu g \cdot L^{-1}$	290 (20)	280 (10)	260	300 (40)	280 (20)	145	180 (20)	180 (20)	250	300 (40)	310 (30)	200
Susp. P	$\mu g \cdot L^{-1}$	3.5 (1.0)	3.0 (0.8)	3	3.3 (1.3)	3.0 (0.0)	3	3.5 (1.0)	3.3 (0.5)	3	3.8 (1.0)	3.0 (0.8)	11
TDP	$\mu g \cdot L^{-1}$	4.8 (2.5)	6.8 (2.5)	5	n.d.	n.d.	n.d.	1.1 (0.7)	0.8 (0.4)	n.d.	1.8 (2.2)	1.3 (0.5)	2
DIC	$\mu mol \cdot L^{-1}$	140 (30)	150 (20)	130	150 (10)	140 (0)	130	130 (10)	130 (10)	140	170 (10)	160 (10)	140
DOC	$\mu mol \cdot L^{-1}$	690 (20)	680 (20)	700	740 (20)	730 (10)	700	700 (10)	700 (10)	720	750 (50)	730 (20)	690
Susp. C	$\mu g \cdot L^{-1}$	570 (100)	550 (40)	620	580 (60)	660 (20)	890	670 (80)	630 (50)	780	640 (50)	610 (40)	1260
Chl *a*	$\mu g \cdot L^{-1}$	-	-	2.26	1.4 (0.4)	2.1 (0.1)	-	2.3 (0.5)	2.3 (0.2)	3.1	2.3 (0.5)	2.2 (0.5)	23.8
pH		-	-	6.94	7.2 (0.1)	7.2 (0.1)	7.00	7.1 (0.1)	7.1 (0.1)	7.0	7.2 (0.0)	7.1 (0.1)	7.1
O_2	$mg \cdot L^{-1}$	-	-	-	-	-	5.80	6.0 (0.7)	5.7 (0.4)	-	8.5 (0.5)	7.9 (0.4)	7.4

Discussion

Epilithic metabolism

The agreement between our experimental results and our long-term data analysis demonstrates that epilithic photosynthesis can be stimulated by temperature, and that this may be an important force *in situ*. Although light may be correlated with temperature in the long-term data, epilithic algae are not typically light limited in L239 at our study depths (Turner et al., 1991), so the observed temperature correlation was probably independent of irradiance. These findings are consistent with a seasonal study by Gruendling (1971) who showed that light, temperature and biomass were the primary controls on seasonal changes in epipelic (sediment-associated biofilm) productivity in Marion Lake, BC. Our findings also agree with those of Hickman and Klarer (1975) who found that epiphytic (plant-associated biofilm) gross primary production was stimulated by thermal pollution. However, Hickman and Klarer attributed this increased production to increases in biomass, measured as chlorophyll *a*. Given that algae may acclimate to increased temperatures by increasing their pigment content (Huner et al., 1998), this interpretation should be revisited.

A greater amount of research has addressed the influence of temperature on photosynthesis of stream communities. In artificial streams Kevern and Ball (1965) found no significant effect of temperature on net productivity when they increased water temperatures by 5.6°C. However, incident light was approximately 1% of natural levels, indicating algae in this study were probably light limited. In contrast, Fuller (unpublished data in DeNicola, 1996) found that rates of net primary production normalized to biomass increased significantly when the water temperature in artificial streams was raised from 5°C to 12°C. Similarly, Phinney and McIntire (1965) measured changes in oxygen concentration in a laboratory stream and found that rates of net photosynthesis were stimulated by a 10°C increase in temperature at saturating light intensities. DeNicola's (1996) meta-analysis also supports the hypothesis that rates of net photosynthesis of benthic algae in streams are temperature dependent. Maximal rates of photosynthesis increased exponentially with increasing temperature (DeNicola, 1996).

Rates of photosynthesis within the epilithic community may be further affected by changes in concentrations of DIC associated with climate change. Epilithic algae are DIC-limited within L239 (Turner et al., 1994), therefore changes in DIC availability are expected to affect photosynthetic rates. Schindler et al. (1996), found that alkalinity increased in L239 during a period of unusually warm and dry period from 1970-1990. This was due to an increase in the water residence time, a decrease in precipitation, an increase in calcium concentrations and an increase in sulfate removal. However, the period from 1995-2000 that was characterized by warm water and high precipitation, showed a decline in alkalinity (R. Hesslein, personal communication). Therefore, changes in precipitation are important to the DIC response. Finally, rates of transport of DIC into the benthic boundary layer may limit DIC availability. Changes in wind and wave action, as well as changes in thermal convection may affect DIC availability and in turn may affect rates of epilithic photosynthesis.

Rates of light saturated photosynthesis were elevated by the heat treatment, probably due to enhanced carbon fixation associated with increased enzymatic activity, enhanced rates of ATP regeneration or accelerated transport processes (Davison, 1991). Our results for epilithon thus agree with numerous studies of marine and freshwater phytoplankton (Platt and Jassby, 1976; Collins and Boylen, 1982; Lindström, 1984; Post et al., 1985; Levasseur et al. 1990; Iriarte and Purdie, 1993; Rae and Vincent, 1998; Coles and Jones, 2000), as well as with several studies of benthic species (Mantai, 1974; Hawes, 1993; Graham et al., 1996; Blanchard and Guarini, 1997; Tang and Vincent, 1999) that showed increased maximal photosynthetic rates with increased temperatures within an optimal range.

The lack of a temperature effect on the initial slope of the photosynthesis-irradiance curve in this study was also seen in numerous studies of phytoplankton (Platt and Jassby, 1976; Post et al., 1985; Iriarte and Purdie, 1993; Rae and Vincent, 1998), and a study of epilithon in a laboratory stream (Phinney and McIntire, 1965). Light limited-photosynthesis is considered a temperature-independent process limited by the rates of photochemical reactions (Steemann Nielsen and Jørgensen, 1968). However, Davison (1991) contends that the temperature dependence of enzymes active in photosynthesis could lead to temperature dependence of light-limited photosynthesis, a result also supported by a number of studies (Fawley, 1984; Levasseur et al., 1990; Tang and Vincent, 1999; Coles and Jones, 2000). The current study indicates that the effects of warming on rates of light-limited photosynthesis are unlikely to be large, although results are not conclusive due to the use of only two replicates, and the limited number of light treatments studied.

Our exchange experiments provide insight into whether the increase in rates of net photosynthesis associated with warming was a direct metabolic response or whether changes were driven by temperature-related changes within the epilithon. If higher pigment concentrations were to confer greater photosynthetic capacity to warm acclimated tiles, these tiles would show higher photosynthetic rates than cold acclimated tiles at high and low temperatures. However, increases in photosynthetic rates were observed only when tiles were incubated at the higher temperature, which suggests that increased pigment content conferred greater photosynthetic capacity only at higher temperatures. Alternatively a shift in taxonomic composition to species with higher optimal temperatures might also have been expected to drive this community related effect; however, algal counts did not show any significant taxonomic change (Chapter 4). Finally, the convergence of photosynthetic rates in control enclosures irrespective of colonization history and the difference between metabolic rates of both warm and control enclosure communities at different temperatures, indicate that direct metabolic effects of increased temperature were the dominant control on photosynthetic rates.

Rates of community dark respiration increased as a result of enclosure warming as expected, given that respiration rates of algae (Collins and Boylen, 1982; Iriarte and Purdie, 1993; Graham et al., 1996), bacteria (Thamdrup and Fleisher, 1998; Pomeroy and Wiebe, 2001) and invertebrates (Sweeney, 1978; Höckelmann and Pusch, 2000) are known to increase with temperature. This result was also corroborated by the positive correlation between temperature and respiration in the long-term data. Community respiration rates of stream periphyton (Phinney and

McIntire, 1965; Kevern and Ball, 1965) have also been shown to increase with temperature.

Respiration rates were much more dependent on incubation temperature than on colonization history, suggesting that stimulation of metabolic rates in warmed enclosures is chiefly a metabolic effect rather than a result of community change. If the heat treatment induced a shift in algal species, then changes in respiration rates might be expected, because of the possibility that species may differ in their respiration rates, or could show differing respiratory responses to increasing temperatures. However, as already noted, algal counts did not indicate that any significant changes occurred in the algal community (Chapter 4). We expected that increased bacterial abundance in warmed enclosures (Chapter 4) would contribute to greater respiration rates on warm acclimated tiles. However, when warm and control acclimated tiles were incubated in control enclosures, respiration rates of tiles from control enclosures were consistently higher. Because a time × treatment interaction was observed, this cannot be statistically verified. Changes in the invertebrate community could also explain these trends; however, differences in invertebrate abundance between warm and control enclosures were minimal (Chapter 4). When incubations of warm and control acclimated tiles were done in warmed enclosures the expected trend was shown, although results were not statistically significant.

The proportion of fixed carbon allocated to respiration is estimated by the ratio R_{dark}:P_{gross}. Gross photosynthesis may be overestimated by adjusting rates of net photosynthesis for dark respiration because dark respiration rates may differ from rates of respiration in the light (Scherer and Böger, 1982; Graham and Turner, 1987). However, this bias is diminished by inclusion of dark respiration in both the numerator and denominator of the ratio (Turner et al., 1995). The warming-induced increase in the ratio R_{dark}:P_{gross} indicates that a greater proportion of assimilated carbon is lost to respiration, a common indicator of stress (Odum, 1985; Schindler, 1990). The lack of effect on the first two dates is interesting, and may reflect error related to measuring R_{dark} and P_{gross} on different dates, or could be due to a greater sensitivity of the community to increased temperatures when ambient water temperatures are lower. However, the long-term data analysis showed a correlation between temperature and R_{dark}:P_{gross} across a wide range of temperatures. In benthic communities of a desert stream, respiration rates were also more highly temperature dependent than photosynthetic rates, and an increase in R_{dark}:P_{gross} was observed at elevated temperatures (Busch and Fisher, 1981). Extrapolating R_{dark}:P_{gross} measurements to longer timescales is inappropriate because stimulation of photosynthetic rates is restricted to daylight hours but respiration rates may be stimulated in both the light and dark. Diurnal as well as seasonal temperature changes will further affect the balance.

Reduced water movement probably inhibited photosynthesis in metabolic chambers. Turner et al. (1991) found that epilithic photosynthetic rates in static chambers (mean benthic boundary layer thickness = 580 µm) were only one-third of rates in re-circulating chambers that had a mean boundary layer thickness of 210 µm. The thickness of the benthic boundary layer within enclosures was approximately 230 µm (Chapter 2), hence conditions within enclosures would have been better approximated by the use of re-circulating chambers. Boundary layer thickness within enclosures was within the range of measurements in the lake (Chapter 2), and although boundary layer thickness in warmed enclosures was 2-4% greater than in controls during the daytime (Chapter 2), this difference was likely too small to significantly affect photosynthetic rates, and was not statistically significant.

Pigment concentrations

Ratios of chlorophyll *a* and total carotenoids to algal biomass were consistently higher in warmed enclosures; however, results were not statistically significant. A number of studies of cultured algae have shown temperature-dependent increases in cellular chlorophyll *a* quotas (Kübler and Davison, 1995; Coles and Jones, 2000). However, results were not uniform and may depend on the species of interest (Morris and Glover, 1974; Coles and Jones, 2000) or cultivation light intensity (Post et al., 1985). Chlorophyll *a* concentrations may also be affected by temperature in a non-linear manner (Morris and Glover, 1974; Cloern, 1977;

1977; Goldman and Mann, 1980), or concentrations may be unaffected by temperature (Levasseur et al., 1990).

Ratios of pigments to carbon are poorer indicators of biomass-specific pigment content because non-algal biotic and abiotic constituents are included in carbon measurements. The significant increases in ratios of pigments to carbon in warmed enclosures were driven by the significant increase in areal concentrations of chlorophyll *a* and carotenoids (Chapter 4). Carbon concentrations were unaffected by the heat treatment (Chapter 4). The analysis of pigment to carbon ratios had higher power than chlorophyll to biomass ratios due to more frequent sampling, and lower variability associated with pooling two replicate carbon values. Increased chlorophyll to carbon ratios have been reported in numerous species of cultured algae at elevated temperatures (Li, 1980; Geider, 1987; Davison, 1991).

Elevated temperatures are expected to stimulate electron transport activity, allowing algal cells to harvest more light without risk of photoinhibition (Maxwell et al., 1995). Increased ratios of chlorophyll *a* and carotenoids to carbon in warmed enclosures may be an adaptation to allow this increased light harvest and facilitate the observed stimulation of maximum photosynthetic rates. In a study of *Laminaria saccarina*, Machalek et al. (1996) found that fucoxanthin and chlorophyll *c* concentrations were considerably higher in plants grown at 17°C than in plants grown at 5°C. Sosik and Mitchell (1994) showed that concentrations of accessory pigments increased with temperature in *Dunaliella tertiolecta*. Tang and Vincent (1999) found that *Phormidium subfuscum* showed a slight increase in carotenoid content with temperature, whereas the carotenoid content of *P. tenue* varied with temperature, generally declining with increased temperature over the 35°C range studied. The relationship between temperature and algal pigment content is complicated by the role of photoprotective pigments. At low temperatures concentrations of some photoprotective pigments may increase as a protective mechanism against photoinhibition (Young, 1993), which may help explain the temperature responses of *P. tenue*.

Changes in ratios of carotenoids to chlorophyll at elevated temperatures may be driven by accelerated degradation of chlorophyll *a* or changes in cellular carotenoid concentrations. Chlorophyll *a* is more susceptible than some accessory pigments to accelerated degradation at high temperatures (Jen and Mackinney, 1970; Young, 1993). Increased grazing pressure expected in warmed enclosures (Deason, 1980; Dumont and Schorreels, 1990), could also lead to accelerated chlorophyll degradation (Daley, 1973; Daley and Brown, 1973). However, the lack of significant effect of the heat treatment on pheophytin to chlorophyll ratios suggests that chlorophyll *a* degradation was not significantly affected. Similarly, there was no effect of the heat treatment on carotenoid to chlorophyll ratios, indicating that any change in carotenoid content was roughly proportional to changes in chlorophyll content. Other researchers have found that ratios of accessory pigments to chlorophyll may increase (Tang and Vincent, 1999; Ivanov et al., 2000), decrease (Sosik and Mitchell, 1994; Maxwell et al., 1994; Ben-Amotz, 1996; Tang et al., 1997), or remain static with increasing temperature (Machalek et al., 1996; Tang et al., 1997; Tang and Vincent, 1999).

Biomass

Increased temperatures have been associated with increased algal growth rates (Eppley, 1972; Li, 1980) and higher algal biomass (Gruendling, 1971). However, effects may depend on the magnitude of the change in temperature, the nutrient or light status of the community (Rhee and Gotham, 1981; Bothwell, 1988), the long-term mean temperature of the environment (Patrick, 1971), or the maturity of the epilithon (Rempel and Carter, 1986). Algal biomass is a function of photosynthesis, respiration, carbon exudations, and removal processes including grazing and wave scouring. Despite the significant stimulation of rates of net photosynthesis in warmed enclosures, there was no difference in algal biomass between warm and control enclosures. This apparent uncoupling between growth and photosynthesis may be due to increased carbon exudations as seen by Collins and Boylen (1982) in the Cyanobacteria *Anabaena variabilis*. They showed that the percent of assimilated carbon released as dissolved organic carbon (DOC) varied with temperature at low light levels. At moderate light levels, DOC release was stimulated at temperatures above 35°C and at high light levels DOC release increased markedly only at 40°C.

This suggests that if carbon exudations were stimulated by the heat treatment, this effect would be most noted under low light conditions, and perhaps at night. However, there is no evidence of enhanced DOC release at moderate temperatures (Collins and Boylen, 1982), suggesting that this mechanism is not important in our experiment. Extrapolation of measurements of net photosynthesis to longer time-scales may also be inappropriate. If respiration rates are stimulated at night, the carbon gain in the daytime may be negated by accelerated loss of carbon at night. Finally, warming is associated with increased metabolic rates of invertebrates (Sweeney, 1978; Höckelmann and Pusch, 2000). This probably led to increased grazing pressure within warmed enclosures that limited biomass accrual, and likely contributed to the lack of a biomass effect. Although the ratio of pheophytin to chlorophyll *a* may be affected by grazing, the absence of effect on this ratio does not preclude the occurrence of changes in grazing rates. A multitude of factors affect both the synthesis and breakdown of chlorophyll *a*.

Artificial substrata and enclosure effects

Rates of net photosynthesis and dark respiration on tiles were higher than rates on the natural bedrock substrata. Although metabolic incubations on bedrock were done in slightly deeper water than tile incubations, Turner et al. (1983, 1991) have shown that epilithon is not likely to be light limited at our study depth, hence the depth difference is not adequate to explain the discrepancy. Instead, this may be a substratum-effect or it may reflect differences in self-shading between the mature rock community and the developing tile community. Although taxonomic composition of algal communities frequently differs between natural and artificial substrata (Tuchman and Stevenson, 1980; Barbiero, 2000), our communities were qualitatively similar. The only major taxonomic difference was that *Lyngbya* abundance was greater on tiles than on the natural substrata (D. Findlay, Freshwater Institute, personal communication). Nonetheless, the limitations of artificial substrata must be considered when interpreting results of this study.

In addition, enclosure effects must be considered. Although ecosystem-level warming experiments are currently feasible only in small-volume systems (Hogg and

Williams, 1996), whole-ecosystem experimentation is necessary to fully understand complex ecological effects over large spatial scales and longer time periods (Schindler, 1998). In this case, enclosure studies should be considered a major advance, as the only lake-scale temperature manipulations have involved changes in the light environment (Klarer and Hickman, 1975), erosion control (Patalas, 1970; Konopacka and Jesionowska, 1995), introduced species (Patalas, 1970; Konopacka and Jesionowska, 1995) or high temperature effluents that poorly simulate climate-change scenarios (Gallup and Hickman, 1975). However, limitations, including those specific to our experimental apparatus must be considered. Effects on boundary layer thickness at night, and differences in the biofilm on pipes in warm and control enclosures were observed (Chapter 2). Controlled lake warming experiments may be achievable in the future if hot water effluents are used in a closed circulation heat-exchange system. Until this is feasible, small-scale experiments and long-term data analyses must serve as surrogates, although their limitations must be recognized.

Conclusions

Increased water temperatures comparable with a climate change scenario resulted in increased rates of epilithic photosynthesis, respiration, and an increase in the ratio of R_{dark}:P_{gross} near the end of the experiment. Temperature and metabolic parameters were also correlated in long-term data. Increased rates of net photosynthesis in warmed enclosures were driven by stimulation of light-saturated photosynthesis. Stimulation of photosynthetic rates is thought to be related to accelerated enzyme activity and electron transport; however, observed increases in pigment concentrations may have contributed to improved light harvesting efficiency. Higher water temperatures also led to increased rates of community respiration and resulted in an increase in the proportion of fixed carbon lost to respiration near the end of the experiment. Algal biomass did not increase in conjunction with increased net photosynthesis in warmed enclosures, indicating that increased grazing, accelerated night time respiratory carbon loss or increased carbon exudations negated the effects of photosynthetic stimulation.

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Chapter 4: Effects of warming on epilithic community composition

Introduction

Humans have altered the thermal regime of rivers, ponds and lakes by diverting water, creating impoundments, harvesting forests, and releasing heated effluents. While these alterations have generally been local in scale, the threat of global warming is a nearly ubiquitous one. Average annual air temperatures in North America are predicted to increase by 1.4 to 5.8°C between 1990 and 2100 (Intergovernmental Panel on Climate Change, 2001). Corresponding to this change, surface temperatures of stratified lakes in the Precambrian Shield and Laurentian Great Lakes regions are expected to rise by 1-7°C (Magnuson et al., 1997).

Relatively little is known about the climatic responses of littoral communities, despite their importance as an energy source to higher trophic levels (Hecky and Hesslein, 1995). Epilithon is rock-associated community within the littoral zone that consists of detritus, algae, fungi, bacteria and invertebrates. We focussed our research on the response of epilithon to an increase in water temperatures of 4.5°C. Specifically, we measured changes in carbon accrual, community nutrient ratios and changes in the algal, bacterial and invertebrate communities.

The taxonomic composition of benthic algae may be affected by light, nutrient availability, pH, competition, grazing, wave action, and temperature. Typically, benthic algae are dominated by diatoms at temperatures below 20°C, chlorophytes at temperatures between 15-30°C, and Cyanobacteria at higher temperatures (reviewed by DeNicola, 1996). The abundance of chlorophytes, and in particular *Mougeotia* spp., is positively related to temperature (Schindler et al., 1990; Turner et al., 1995a). The shift from diatoms to chlorophytes and Cyanobacteria at increased temperatures is expected to lead to reduced algal richness and diversity (reviewed by DeNicola, 1996). In addition, increases in algal biomass may be associated with higher water temperatures (Gruendling, 1971), although this response may be moderated by increases in grazing rates (Deason, 1980; Dumont and Schorreels, 1990).

The use of algal pigments to infer taxonomic composition in the oceans is widespread. Increasingly, pigments are also being used in freshwater benthic and pelagic communities (e.g. Schmid et al., 1998; Vinebrooke and Leavitt, 1999). This method has shown good agreement with direct microscopic enumeration for freshwater phytoplankton (Leavitt and Findlay, 1994; Schmid et al., 1998). However, in benthic communities, factors that can affect algal pigment composition including light, species composition and nutrient availability (Rosen and Lowe, 1984; Deventer and Heckman, 1996; Schofield et al., 1998) may be more spatially variable. Poorer correlation between taxonomic composition and pigments has been shown in the benthos, possibility as a result of greater environmental heterogeneity (Havens et al., 1999). We paired our investigation of algal taxonomy with the use of taxonomically diagnostic pigments to determine whether changes in water temperature affected the reliability of the pigment results. We expected that increased pigment content of algal cells acclimated to warmer water (Sosik and Mitchell, 1994; Machalek et al., 1996) would contribute to a decoupling of the results of these two methods.

Increased temperatures are expected to directly stimulate bacterial growth rates (Edwards and Meyer, 1986) leading to increased bacterial abundance (Lamberti and Resh, 1983; Osborne et al., 1983). Warming may also indirectly affect bacterial abundance. For example, increased algal biomass resulting from warming may increase the amount of substrate available to bacteria. In addition, stimulation of grazing rates by increased water temperatures (Deason, 1980; Dumont and Schorreels, 1990) could directly reduce bacterial abundance, but could also increase the availability of dissolved organic matter to bacteria and lead to an increase in bacterial abundance (del Giorgio and Scarborough, 1995; Møller and Nielsen, 2001). Temperature and substrate availability may also have interactive effects, as have been observed in oceans, and lakes (Pomeroy et al., 1991; Wiebe et al., 1992; Felip et al., 1996). The average size of bacterial cells may decline with warming (Atkinson, 1994); however, results in the literature are contradictory (Chrzanowsi et al., 1988; Ben-Dan et al., 2000).

Studies of thermal pollution and controlled experimental studies have shown that warming leads to a decline in the abundance of benthic macroinvertebrates

(Lamberti and Resh, 1983; Hogg and Williams, 1996; Ferguson and Fox, 1978) and meiofauna (Oden, 1979). Declines in the abundance of Ephemeroptera, Hemiptera, Odonata and Trichoptera have been observed in warmed areas (Ferguson and Fox, 1978), while responses of dipterans have been variable (Dusoge and Wisniewski, 1976; Ferguson and Fox, 1978; Hogg and Williams, 1996). Increased water temperatures may also accelerate invertebrate development (reviewed in Arnell et al., 1996; Wilhelm and Schindler, 2000), stimulate grazing rates (Deason, 1980; Dumont and Schorreels, 1990) and affect fecundity (Folsom and Clifford, 1978; Sweeney, 1978; Wilhelm and Schindler, 2000).

Temperature could affect epilithic nutrient ratios via changes in algal nutrient quotas (Goldman 1979; Rhee and Gotham 1981; Raven and Geider, 1988; Thompson et al., 1992; Thompson, 1999), nutrient uptake rates (Cloern, 1977; Rhee and Gotham, 1981), rates of carbon fixation (Chapter 3), or growth rates (Chalup and Laws, 1990). The temperature dependence of nitrogen to phosphorus (Goldman, 1979) and carbon to nitrogen ratios (Thompson et al., 1992) has been illustrated in culture studies of single algal species, but has not been shown at a community level. Changes in epilithic nutrient ratios could also reflect changes in the abundance or nutrient content of bacteria, invertebrates or detritus.

To evaluate warming effects on the epilithon, we warmed four littoral enclosures by 4.5°C while maintaining four others as controls. We investigated effects on algae, bacteria, and invertebrates in addition to effects on carbon accrual and food quality:

1. We expected to observe a decline in biomass of diatoms with warming, paired with an increase in the biomass of chlorophytes, and an increase in overall algal biomass. The increase in chlorophytes and decrease in the abundance of species-rich diatoms was expected to lead to a loss of algal richness and diversity in warmed enclosures (DeNicola, 1996). Further, we predicted that the size of algal cells would decline in warmed enclosures, consistent with Atkinson's (1994) size-temperature hypothesis.

2. We hypothesized that bacterial abundance would increase (Lamberti and Resh, 1983; Osborne et al., 1983), but cell size would decline (Atkinson, 1994). We predicted the overall effect would be an increase in total bacterial biovolume.
3. We expected that a decline in invertebrate abundance (Oden, 1979; Lamberti and Resh, 1983) and a loss of sensitive species (Ferguson and Fox, 1978; Oden, 1979) would to lead to reduced richness and diversity in warmed enclosures (Logan and Maurer, 1975; Oden, 1979). In addition, we anticipated that warming would accelerate rates of invertebrate development (Sarvala, 1979; Abdullahi, 1990).
4. We monitored changes in nutrient ratios, expecting that changes in algal nutrient composition (Goldman, 1979; Rhee and Gotham, 1981; Raven and Geider, 1988) could drive a change in community level stoichiometry. In conjunction with food quality, we anticipated food quantity, roughly approximated here as areal carbon accrual, would increase as a result of increased algal growth and increased bacterial abundance.

We investigated changes in algal taxonomy, stoichiometry and carbon accrual on a number of substrata to investigate whether community maturity or substratum type affected the response to warming.

Materials and Methods

Enclosures

To study the effects of a 4.5°C temperature increase on the epilithon, eight 700-L enclosures were constructed along the western shore of Lake 239 (L239) at the Experimental Lakes Area in northwestern Ontario. Half of the enclosures were heated for eight weeks (August 3-September 27, 2000) using a closed circulation heat-exchange system (Chapter 2), while half of the enclosures were maintained as controls.

Enclosures were warmed by circulating hot water through a network of heat exchange pipes (1.3-cm diameter Kitec Al-Pex, Ipex Inc.). Temperature was controlled using an electronic temperature control system that operated a series of solenoid valves (Asco electric). When the sensed temperature difference between a pair of warm and control enclosures was less than 5°C, a valve would open, and hot water would enter the heat exchange pipe installed at the bottom of the warmed enclosure. When the sensed temperature difference exceeded 5°C the valve closed, and water was shunted through a bypass loop. Control enclosures also contained heat-exchange pipe; however, this pipe was not attached to the heating system.

Temperatures were measured every 15 minutes using recording thermocouples (HoboTemp, Onset). The mean temperature difference between warm and control enclosures during the experiment was 4.5°C. Temperatures within control enclosures were similar to adjacent areas of the lake and patterns of natural diurnal variation were maintained in control and warmed enclosures (Chapter 2).

The treatments were randomly assigned to enclosures using a blocking design. Enclosures were 1.4 m x 1.4 m with an open bottom and a 0.7 m skirt that was secured to the lake bottom using sandbags. The sandbags did not provide a complete seal. The mean water residence time was estimated at 5.6 days (determined by sodium chloride addition at the end of the experiment). The bottoms of the enclosures were open to the bedrock substratum to allow colonization of tiles by natural communities.

Artificial and natural substrata

Epilithon was studied on both unglazed ceramic tile and natural bedrock substrata. Before placement, tiles (4.8 cm x 4.8cm) were combusted at 600°C for a minimum of one hour, scrubbed, soaked in dilute hydrochloric acid overnight, rinsed with deionized water and dried. To minimize differences between the tile community and the natural epilithon, tiles were pre-colonized in L239 for eight weeks prior to the start of the experiment. Tiles were placed a minimum of 10 cm away from the heat exchange pipes within enclosures to minimize effects of the pipe community on study communities (Chapter 2). Sampling of pre-colonized tiles was often divided over several days, but enclosures within a single block were always sampled at the same time (Chapter 3). Tiles were selected using a matrix of random numbers corresponding to tile distribution, and slowly transferred into a plastic tray that was gently placed within an acrylic chamber. Chambers were sealed, and kept chilled until processing when the epilithon was scraped into 1 L of lake water using plastic scrapers.

The natural bedrock substratum was sampled within enclosures to assess warming effects on this community. Samples of epilithon were obtained from enclosure areas that were not visibly disturbed and were located a minimum of 10 cm from the heating pipes. In addition, we sampled the bedrock community outside of the enclosures at depths of 0.49-0.62 m to allow us to compare carbon accrual and nutrient ratios in this natural community to the tile community. Samples of epilithon were obtained from rocks using a scraping brush sampler (Turner et al., 1987) and three or four scrapings (5 cm^2 per scraping) from each sampling site were combined.

Epilithic suspensions were blended for 10 s at low speed to homogenize the samples, transferred to stirred beakers, and subsampled using a wide-bore syringe. Two duplicate suspensions from each enclosure were sampled for pigment and nutrient analyses, and the mean of these two values was used in analyses.

Colonization experiment

We performed a colonization experiment to determine whether warming effects on early successional communities differed from effects on more mature communities. We placed bare tiles in the enclosures on August 19th and allowed them to colonize for four weeks. After four weeks, tiles were harvested and sampled as previously described. These tiles are termed short-term colonized (STC) tiles.

Algal taxonomy

To determine how the heat treatment affected algal community composition, richness and diversity, subsamples of the epilithic slurry were obtained using a wide-bore syringe, preserved with acid Lugol's (4% final concentration) and analysed using the modified Utermöhl technique (Nauwerck, 1963). Samples were sonicated at 20 kHz (Sonifer Cell Disruptor, Model W140, Heat Systems, Ultrasonic Inc.) for two fifteen-second intervals. Subsamples were stained with Fast Green FCF (Fisher Scientific) and allowed to settle overnight. Cells were identified to the lowest taxonomic unit using a phase-contrast inverted microscope at 125x and 400x magnification until a minimum of 100 cells of the dominant taxon were counted. Only viable cells that showed the presence of cellular structures were enumerated (Owen et al., 1978). The Shannon-Wiener index (calculated with log base 10) was used as an indicator of species diversity (Krebs, 1989). Preserved subsamples of the rock community within enclosures were examined to allow qualitative comparison of the tile community within enclosures to the community present on the natural substratum.

In each sample, fifty cells of each of the most common taxa were measured and estimates of algal cell or colony biovolume were obtained using regressions for different taxa (Vollenweider, 1974). For less common taxa, cells were measured as they were encountered, and estimates of cell size are based on less than fifty measurements. For brevity, although measurements of algal size include both cells and colonies, we will refer to these measurements as algal cell size. Estimates of algal wet biomass were obtained from algal biovolume measurements (Nauwerck, 1963).

We first assessed warming effects on the composition of the algal community on pre-colonized tiles using a repeated-measures (RM), randomized-block (RB), multivariate ANOVA (MANOVA). Analyses were restricted to the nine dominant algal species that were present on all three sampling dates. A significance level of α=0.05 was selected for all analyses, and all analyses were performed using SYSTAT 8.0. Because the multivariate test showed no significant treatment or time × treatment effects, these effects within individual tests (RM-RB-ANOVAs) were compared to a more conservative Dunn-Šidák adjusted *p*-value (Johnson, 1998). A second RM-RB-MANOVA assessed warming effects at the group level. Results of associated RM-RB-ANOVAs should also be compared to a Dunn-Šidák adjusted *p*-value. Warming effects on *Mougeotia* were analyzed on the final date using a RB-ANOVA. Effects of the heat treatment on the algal community of the STC tiles were first assessed using RB-MANOVAs for groups and species, then with individual RB-ANOVAs. Data were log or power transformed to meet assumptions of all analyses (Taylor, 1961). Effects of the heat treatment on algal richness, diversity and cell size on the pre-colonized tile community were analyzed using RM-RB-ANOVAs, and effects on the STC tile community were analyzed using RB-ANOVAs. We also assessed warming effects on the mean size of five taxa that were present in a minimum of seven enclosures. Due to missing data, a MANOVA could not be run, so results of RB-ANOVAs for each taxon were compared to a Dunn-Šidak adjusted *p*-value.

Pigment analyses

Subsamples of the epilithic slurry (10-20 mL) were filtered onto GF/C filters and frozen prior to analysis for pigment concentrations. Samples were freeze-dried for 24 h at 100 millitor with the specimen chamber at 0°C and the condenser chamber at −40°C (Virtis Model 24DX48 Specimen Freeze Dryer). Dried samples were then extracted in 80% acetone, 15% methanol and 5% water solution (by volume) for 24 h at 10°C in the dark. Extracted samples were filtered through a 0.2-μm membrane filter, dried under nitrogen gas and dissolved in a known volume of injection solvent (70% acetone, 25% ion-pairing reagent, 5% methanol by volume) containing the 3.2

mg L^{-1} of the internal standard Sudan II. Pigments were separated on a HPLC Model 1100 with inline diode array detector and fluorescence detector and a 10-cm Varian Microsorb C18 column with 100-angstrom beads. Taxonomically diagnostic pigments were compared with direct microscopic counts to confirm presence of taxa. Table 4.1 shows the pigments that were detected and corresponding algal taxa found in the study community. Pigment accrual on the pre-colonized tiles was first analysed using a RM-RB-MANOVA, then with individual RM-RB-ANOVAs. Where the sphericity assumption was not met in individual analyses, Huynh-Feldt corrected *p*-values are reported. Pigment to biomass ratios on the pre-colonized tiles were reciprocal transformed and analysed using a RM-RB-MANOVA. Because the overall MANOVA was not significant for treatment or time × treatment effects, results of RM-RB-ANOVAs should be compared to Dunn-Šidak adjusted *p*-values. On the STC tiles we analyzed pigment to biomass ratios and pigment accrual first using a RB-MANOVA, then with individual RB-ANOVAs. Results of individual RB-ANOVAs for pigment to biomass ratios on the STC tiles should also be compared to Dunn-Šidak adjusted *p*-values.

Table 4.1: Algal groups associated with pigments in epilithic samples

Pigment	Group
Chlorophyll *a*	All algae
Chlorophyll *b*	Chlorophytes
Pheophytin *a*	All algae (degradation product)
β-carotene	All algae
Fucoxanthin	Diatoms
Violaxanthin	Chlorophytes
Lutein/Zeaxanthin	Chlorophytes, Cyanobacteria
Canthaxanthin	Cyanobacteria

Nutrient analyses

We filtered subsamples of the epilithic slurry onto pre-ashed GF/C filters for nutrient analyses. Samples for carbon and nitrogen analyses were dried, then frozen and analyzed using a CHN Control Equipment Corporation 440 Elemental Analyzer.

Phosphorus subsamples were frozen, ashed at 500°C for one hour, digested in 1N hydrochloric acid at 104°C for two hours and analysed using the ascorbic acid-molybdate reaction (modified from Stainton et al., 1977). Carbon accrual on the STC tiles and the bedrock substratum within enclosures was analyzed using RB-ANOVAs, and accrual on the pre-colonized tiles was analyzed using a RM-RB-ANOVA. A RM-RB-MANOVA and individual RM-RB-ANOVAs were used to probe warming effects on stoichiometry of the pre-colonized tiles. Warming effects on stoichiometry on the STC tiles and bedrock substratum were assessed using RB-MANOVAs and RB-ANOVAs. Because the overall MANOVAs for the STC tiles and bedrock were non-significant for treatment or time × treatment effects, results of RB-ANOVAs were compared to Dunn-Šidák adjusted *p*-values. We also tested the hypothesis that nutrient ratios and carbon accrual differed between the pre-colonized tiles and natural bedrock substratum outside of the enclosures. This hypothesis was tested using one-sample *t*-tests.

Invertebrates

On September 19, 2000 we removed 40 tiles (921 cm^2 in total) from each enclosure for invertebrate sampling. Tiles were obtained as described previously, scraped with plastic rulers and samples were preserved in 6% formaldehyde. Samples were sieved through a 77-μm sieve and counts were performed until approximately 200 microcrustaceans were identified in each sample. The sample was then sieved through a 500-μm sieve and counted entirely for amphipods, annelids, snails and large insects. Invertebrate diversity was calculated using the Shannon-Wiener index (log base 10; Krebs, 1989). We analyzed warming effects on invertebrate abundance, and ratios of adult copepods to stage 3-5 copepodites using RB-MANOVAs and RB-ANOVAs. Results of RB-ANOVAs were compared to Dunn-Šidak adjusted *p*-values. Effects of the heat treatment on invertebrate richness and diversity were assessed using RB-ANOVAs.

Bacteria

Bacterial samples were preserved in 2% formaldehyde and stored at 4°C until counts were performed. Samples were homogenized by sonicating for 15 one-second intervals (Vibra cell, 15 W) following the addition of sodium pyrophosphate (final concentration 0.175 g • L^{-1}). Subsamples were taken using a wide-bore pipette, and the diluted sample was stained with 0.49 μg • mL^{-1} DAPI for 20 minutes and filtered onto 0.22-μm black polycarbonate filters (Osmonics Inc.). Filters were mounted and enumerated under UV light. A minimum of 300 cells and 10 randomly selected fields of view were enumerated. Fifty randomly selected cells in each sample were measured using an adjustable ocular micrometer for calculation of cell biovolume. A RM-RB-MANOVA was used to assess warming effects on bacterial density, cell size and total biovolume on the pre-colonized tests. Because the MANOVA was not significant for treatment or time × treatment effects, results of RM-RB-ANOVAs were compared to Dunn-Šidak adjusted *p*-values.

Results

Algal taxonomy

On the pre-colonized tiles, diatoms dominated the algal community in both warm and control enclosures, contributing 51-85% of total algal biomass during the experiment. Chlorophytes and Cyanobacteria comprised the balance of algal biomass (Table 4.2). Multivariate tests for effects of the heat treatment on the biomass of algal groups and nine dominant species showed no effects of the heat treatment or of a time × treatment interaction. Biomass of several taxa did vary over time (Table 4.3). At the end of the experiment, the mean biomass of *Mougeotia* was more than ten-fold higher in warmed enclosures than in controls. This genus was not found in one block of enclosures, making the effect statistically non-significant (RB-ANOVA, F=6.27 p=0.09).

Algal taxonomic composition on the short-term colonized (STC) tiles was significantly affected by the heat treatment. There was a seven-fold increase in diatom biomass accompanied by a near doubling in biomass of Cyanobacteria and an almost four-fold increase in the biomass of chlorophytes (Table 4.4). The increase in diatom biomass was driven by a more than 20-fold greater biomass of *Rhopalodia* in warmed enclosures than in control enclosures (Table 4.4). The chlorophytes *Mougeotia* and *Oedogonium* also increased in biomass in warmed enclosures, but results were not statistically significant (Table 4.4). *Lyngbya* biomass increased as a result of warming, but there was no effect of the heat treatment on the Cyanobacteria *Chroococcus limneticus* (Table 4.4). The stronger increase in diatoms caused a decline in the proportion of biomass contributed by both chlorophytes and Cyanobacteria.

The algal community on tiles generally was representative of the natural bedrock community; however, *Lyngbya* abundance was greater on tiles than on the natural rock substratum (D. Findlay, personal communication).

Table 4.2: Effect of the heat treatment on biomass of algal taxa present on pre-colonized tiles. The five species in each group with the greatest contribution to total biomass are shown. Mean biomass ($\mu g\ cm^{-2}$) in four enclosures is shown with the standard deviation indicated in brackets (n.d. indicates the taxon was not detected). Data are from two days after the initiation of the heat treatment to four days before termination of the experiment.

	August 5		August 25		September 23	
	Control	Warm	Control	Warm	Control	Warm
Cyanobacteria	61.3 (7.4)	77.4 (19.3)	104.1 (12.4)	125.2 (50.8)	154.9 (78.0)	124.1 (51.2)
Lyngbya sp.	50.5 (6.1)	62.7 (23.4)	82.6 (19.5)	108.8 (55.9)	110.1 (29.3)	92.4 (36.1)
Anabaena sp.	3.6 (4.7)	2.6 (3.6)	13.6 (9.6)	7.5 (5.3)	2.1 (2.3)	2.3 (3.2)
Nostoc sp.	n.d.	n.d.	n.d.	n.d.	29.9 (59.8)	n.d.
Anabaenaopsis sp.	2.3 (2.0)	5.9 (4.7)	3.9 (4.5)	2.0 (1.4)	3.9 (6.9)	4.8 (5.8)
Scytonema sp.	n.d.	n.d.	n.d.	n.d.	6.9 (13.8)	12.0 (24.1)
Chlorophytes	25.3 (29.4)	22.1 (12.2)	112.1 (172.7)	149.2 (241.2)	82.6 (74.8)	84.1 (54.4)
Bulbochaete sp.	2.8 (5.6)	n.d.	84.6 (169.1)	48.9 (97.7)	n.d.	n.d.
Bambusina brebissonii Kützing	n.d.	n.d.	n.d.	n.d.	67.7 (79.1)	40.4 (46.7)
Oedogonium sp.	12.0 (12.2)	9.0 (8.4)	26.0 (2.4)	23.6 (24.0)	11.8 (17.6)	8.5 (10.2)
Zygnema sp.	n.d.	n.d.	n.d.	76.1 (152.2)	n.d.	n.d.
Mougeotia sp.	1.9 (3.8)	10.1 (18.3)	n.d.	n.d.	2.3 (4.5)	34.0 (26.8)
Diatoms	515.8 (171.5)	574.2 (358.2)	349.2 (178.5)	296.5 (206.5)	302.6 (40.6)	344.8 (156.4)
Rhopalodia sp. O. Muller	424.1 (195.6)	445.1 (331.5)	207.1 (69.4)	175.8 (189.5)	191.5 (48.3)	188.9 (154.8)
Cymbella gracilis (Rabhorst) Cleve	18.5 (15.9)	6.2 (7.2)	56.2 (103.6)	14.0 (16.3)	1.2 (2.4)	20.8 (25.3)
Achnanthes minutissima Kützing	22.8 (9.8)	30.3 (11.7)	16.8 (7.7)	13.4 (6.5)	5.9 (4.9)	9.5 (5.3)
Tabellaria flocculsa (Roth) Kützing	5.3 (4.7)	10.9 (14.0)	5.2 (4.4)	14.2 (13.2)	21.6 (22.9)	33.0 (38.7)
Tabellaria fenestrata (Lyngbye) Kützing	15.3 (14.0)	3.1 (6.2)	1.8 (2.2)	4.6 (7.7)	33.4 (19.6)	28.6 (19.7)

Table 4.3: Results of RM-RB-MANOVAs and RM-RB-ANOVAs assessing the effect of the heat treatment on biomass of three algal groups and on the nine dominant algal species present on the pre-colonized tiles on all three dates studied. The lack of significance of the overall MANOVAs for treatment, and time × treatment effects indicates that these effects should be compared to a more conservative Dunn-Šidák adjusted *p*-value of 0.017 for groups (α=0.05, number of groups = 3) and 0.0057 for species (α=0.05, number of groups = 9).

	Source	df	*F*	*p*
RM-RB-MANOVA (groups)	Heat treatment	1	0.23	0.66
	Time	2	36.9	0.0004
	Time × treatment	2	0.59	0.58
Cyanobacteria	Heat treatment	1	0.14	0.73
	Time	2	23.1	0.002
	Time × treatment	2	3.26	0.11
Chlorophytes	Heat treatment	1	0.33	0.61
	Time	2	0.45	0.66
	Time × treatment	2	0.53	0.62
Diatoms	Heat treatment	1	0.26	0.64
	Time	2	2.77	0.14
	Time × treatment	2	0.15	0.86
RM-RB-MANOVA (species)	Heat treatment	1	3.04	0.18
	Time	2	4.51	0.06
	Time × treatment	2	2.58	0.16
Lyngbya sp.	Heat treatment	1	0.05	0.83
	Time	2	13.6	0.01
	Time × treatment	2	1.97	0.22
Anabaena sp.	Heat treatment	1	2.70	0.20
	Time	2	3.48	0.10
	Time × treatment	2	0.21	0.81
Anabaenaopsis sp.	Heat treatment	1	5.33	0.10
	Time	2	0.23	0.80
	Time × treatment	2	0.29	0.76
Oedogonium sp.	Heat treatment	1	0.64	0.48
	Time	2	2.00	0.22
	Time × treatment	2	0.03	0.97
Rhopalodia sp. O. Muller	Heat treatment	1	0.03	0.87
	Time	2	4.86	0.06
	Time × treatment	2	0.04	0.96
Cymbella gracilis (Rabhorst) Cleve	Heat treatment	1	0.08	0.80
	Time	2	0.28	0.76
	Time × treatment	2	2.79	0.14
Achnanthes minutissima Kützing	Heat treatment	1	1.67	0.29
	Time	2	12.6	0.01
	Time × treatment	2	0.93	0.44
Tabellaria flocculsa (Roth) Kützing	Heat treatment	1	1.78	0.27
	Time	2	5.61	0.04
	Time × treatment	2	0.10	0.90
Tabellaria fenestrata (Lyngbye) Kützing	Heat treatment	1	0.80	0.44
	Time	2	19.7	0.002
	Time × treatment	2	3.07	0.12

Table 4.4: Biomass of dominant taxa in warmed and control enclosures on STC (short-term colonized) tiles after the four-week incubation period. Mean biomass (μg cm^{-2}) in four enclosures is shown with the standard deviation indicated in brackets. (n.d. indicates that the taxon was not detected). Results of RB-ANOVAs assessing the effect of the heat treatment on biomass of dominant taxa present on STC tiles after the four-week colonization period are shown. *Gloeotricia sp., Surirella ovata* and *Aulocoseira binderana* were excluded from analyses because they were present in only one enclosure. 3 df are associated with the each block, and 1 df is associated with the heat treatment. Multivariate tests are reported for the three groups, and for the six species that were analyzed (RB-MANOVA).

	Control	Warm	*F*	*p*
RB-MANOVA (species)			92.6	0.002
RB-MANOVA (groups)			43.7	0.007
Total algal biomass	143.9 (32.5)	669.0 (186.5)	43.6	0.007
Diatoms	71.0 (28.5)	482.7 (188.2)	26.1	0.01
Rhopalodia sp.	14.0 (28.0)	317.8 (54.4)	100.7	0.002
Surirella ovata Kützing	n.d.	55.1 (110.3)		
Aulocoseira binderana Kützing	n.d.	39.6 (79.3)		
Chlorophytes	27.4 (15.2)	103.0 (21.1)	59.1	0.005
Mougeotia sp.	8.0 (16.0)	35.4 (34.5)	1.1	0.37
Oedogonium sp.	1.3 (1.6)	36.4 (25.5)	6.9	0.08
Bulbochaete sp.	15.2 (16.7)	14.5 (18.2)	0.05	0.84
Cyanobacteria	45.2 (5.2)	83.2 (13.2)	22.2	0.02
Lyngbya sp.	36.4 (6.1)	77.4 (16.5)	36.4	0.009
Gloeotrichia sp.	n.d.	3.8 (7.6)		
Chroococcus limneticus Lemmermann	1.8 (2.7)	1.7 (2.5)	0.0006	0.98

Algal richness and diversity

There was no effect of the heat treatment on richness or diversity of algae on the pre-colonized tiles (Table 4.5, Table 4.6). The STC tiles showed a slight decrease in algal diversity as a result of the heat treatment (Table 4.7). Algal richness on the STC tiles was not significantly affected (Table 4.7).

Pigments

A multivariate test indicated that there was a significant time × treatment effect on pigment accrual in the pre-colonized tile communities (Table 4.8). Univariate analyses showed that the heat treatment led to increased accrual of fucoxanthin (diatoms), canthaxanthin (Cyanobacteria), lutein/zeaxanthin (Cyanobacteria, chlorophytes), β-carotene (all algae), chlorophyll *a* (all algae), chlorophyll *b* (chlorophytes), and pheophytin (all algae; Table 4.8, Figure 4.1). Mean violaxanthin (chlorophytes) accrual was generally greater in warmed enclosures, but results were not statistically significant (Table 4.8, Figure 4.1). In contrast, direct microscopic counts showed no change in the biomass of these algal groups (Table 4.3). Accrual of all pigments, excluding β-carotene, varied significantly with time, but a time × treatment interaction effect was not observed for any of the individual pigments (Table 4.8).

Increases in pigment accrual were also observed in the STC tile communities (Table 4.9). Accrual of fucoxanthin, chlorophyll *a*, β-carotene, violaxanthin and canthaxanthin approximately doubled in warmed enclosures. Chlorophyll *b* accrual in warmed enclosures was five-times higher than in control enclosures. Corresponding microscope counts showed greater warming-induced increases in total algal biomass and biomass of some algal groups than indicated by increases in pigment accrual (Table 4.4).

To explore the discrepancy between taxonomic and pigment results, we looked at ratios of pigments to the biomass of taxa indicated by their presence. In heated enclosures, mean ratios of pigments to biomass increased for all taxa on the pre-colonized tiles (Figure 4.2, Chapter 3), although differences were not statistically significant (Table 4.10). In contrast, on the STC tiles, the ratio of chlorophyll *a* to total algal biomass, and the ratio of fucoxanthin to diatom biomass decreased in warmed enclosures (Table 4.11). The same trend was observed for ratios of β-carotene to total algal biomass, chlorophyll *b*

Table 4.5: Algal species richness and the diversity (Shannon-Wiener index) for epilithon on pre-colonized tiles in warmed and control enclosures. The Shannon-Wiener index was calculated using $\log_{10}$. Mean values from four enclosures are shown with standard deviations indicated in brackets.

	Richness		Shannon –Wiener	
	Control	Warm	Control	Warm
Aug. 5, 2000	16.5 (2.4)	15.8 (1.3)	0.78 (0.11)	0.82 (0.06)
Aug. 25, 2000	16.5 (2.4)	14.8 (2.5)	0.86 (0.03)	0.79 (0.10)
Sept. 23, 2000	15.5 (1.3)	16.0 (1.4)	0.71 (0.06)	0.81 (0.11)

Table 4.6: Results of RM-RB-ANOVAs on the effect of the heat treatment on algal species diversity (Shannon-Wiener index) and richness on the pre-colonized tiles.

	Source of variation	df	*F*	*p*
Richness	Heat treatment	1	1.21	0.35
	Time	2	0.12	0.89
	Time × treatment	2	0.50	0.63
Shannon-Wiener	Heat treatment	1	1.33	0.33
	Time	2	2.47	0.17
	Time × treatment	2	3.72	0.09

Table 4.7: Diversity and species richness of epilithon on STC (short-term colonized) tiles in warmed and control enclosures. The Shannon-Wiener index was calculated using $\log_{10}$. Mean values from four enclosures are shown with standard deviations indicated in brackets. Results of RB-ANOVAs are presented. Three df are associated with each block, and 1 df is associated with the heat treatment.

	Control	Warm	*F*	*p*
Richness	19.3 (2.9)	16.5 (2.6)	1.86	0.27
Shannon-Wiener	0.90 (0.05)	0.80 (0.10)	10.4	0.048

Table 4.8: Results of RM-RB-ANOVAs on the effects of the heat treatment on pigment accrual on the pre-colonized tiles. The multivariate test incorporating results for all eight pigments is reported (RM-RB-MANOVA). If the sphericity assumption was not met in individual analyses, Huynh-Feldt corrected *p*-values are reported.

	Source	df	*F*	*p*
RM-RB-MANOVA	Treatment	1	13.9	0.03
	Time	4	36.9	$1.18 \bullet 10^{-6}$
	Time × treatment	4	3.49	0.04
Fucoxanthin	Treatment	1	20.8	0.02
	Time	4	23.1	0.00001
	Time × treatment	4	0.79	0.56
Canthaxanthin	Treatment	1	18.2	0.02
	Time	4	3.75	0.03
	Time × treatment	4	2.29	0.12
Violaxanthin	Treatment	1	4.42	0.13
	Time	4	98.7	$4.48 \bullet 10^{-9}$
	Time × treatment	4	1.20	0.36
Lutein/zeaxanthin	Treatment	1	17.9	0.02
	Time	4	12.2	0.0003
	Time × treatment	4	0.21	0.93
β-carotene	Treatment	1	13.2	0.04
	Time	4	2.98	0.07
	Time × treatment	4	0.86	0.51
Chlorophyll *a*	Treatment	1	28.0	0.01
	Time	4	12.8	0.0002
	Time × treatment	4	0.63	0.65
Chlorophyll *b*	Treatment	1	26.5	0.01
	Time	4	8.58	0.002
	Time × treatment	4	0.62	0.66
Pheophytin	Treatment	1	25.7	0.01
	Time	4	11.2	0.0005
	Time × treatment	4	0.81	0.54

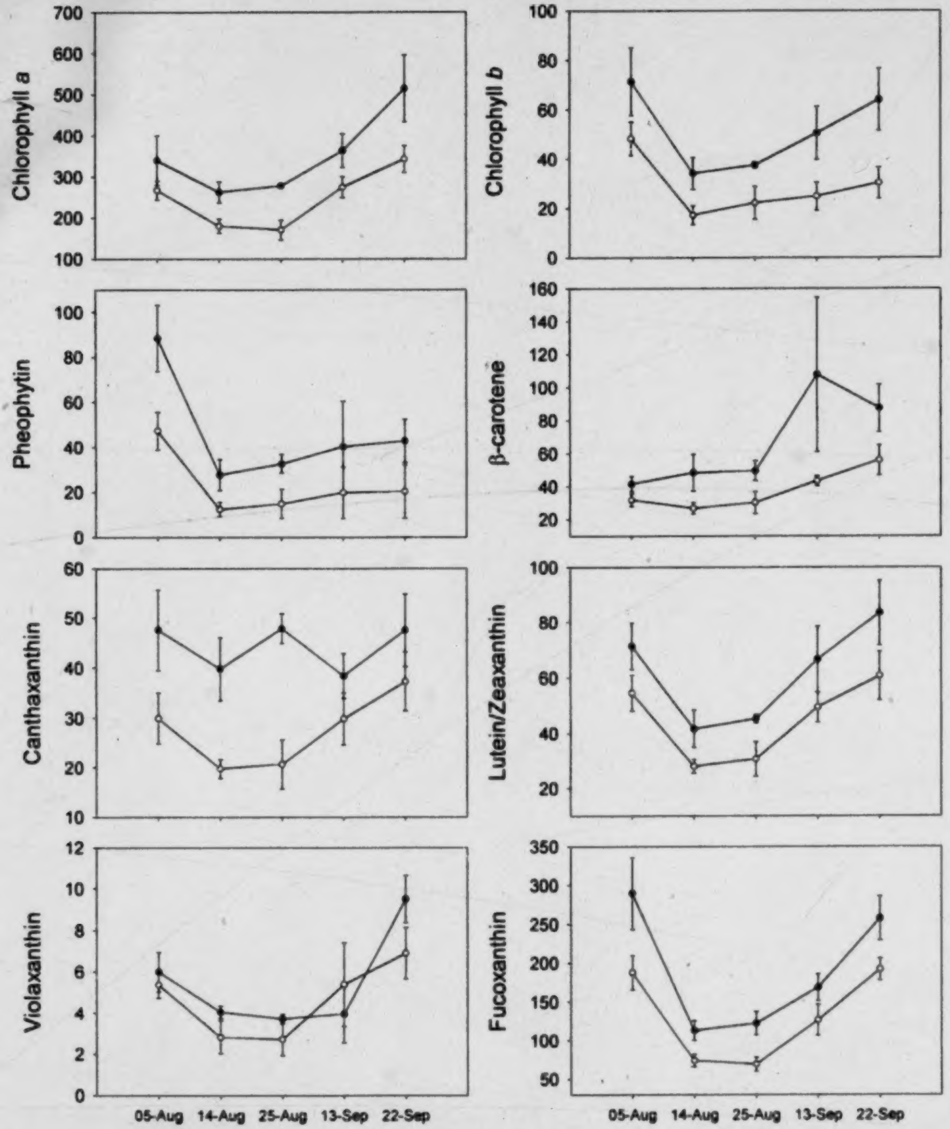

Figure 4.1: Mean pigment accrual (pmol•cm^{-2}) on pre-colonized tiles in control (white circles) and warm (black circles) enclosures. Error bars show ± 1 standard error.

Table 4.9: Pigment accrual (pmol•cm^{-2}) on the STC (short-term colonized) tiles in warm and control enclosures. Data shown are mean values from four enclosures with standard deviations indicated in brackets. The results of a RB-MANOVA assessing the effect of the heat treatment on pigment accrual is shown, and individual tests (RB-ANOVA) are reported.

	Control	Warm	*F*	*p*
MANOVA			39.1	0.01
Chlorophyll *a*	113 (20)	223 (51)	21.6	0.02
β-carotene	16.0 (7.1)	36.0 (16.9)	21.0	0.02
Chlorophyll *b*	8.61 (1.68)	42.7 (23.3)	16.5	0.001
Violaxanthin	4.85 (0.71)	11.5 (9.1)	11.8	0.04
Fucoxanthin	67.6 (21.2)	127 (36)	13.5	0.04
Canthaxanthin	7.43 (2.52)	17.7 (6.5)	114	0.002

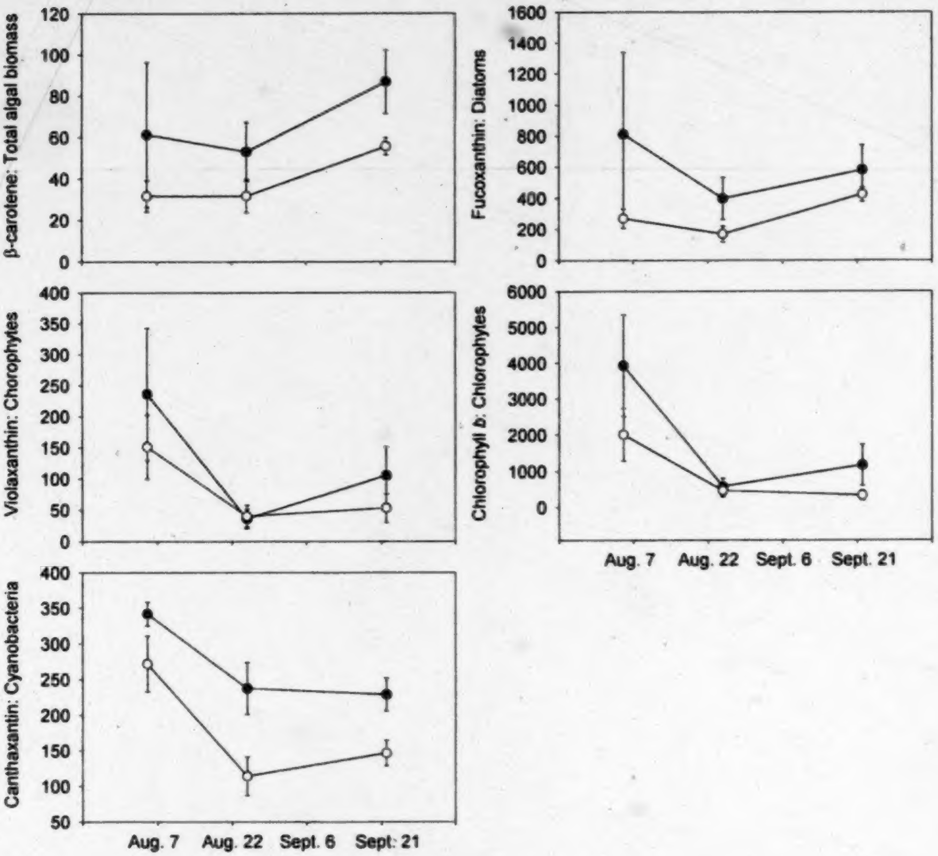

Figure 4.2: Mean ratios of pigments (pg) to algal biomass (μg) on the pre-colonized tiles in control (white circles) and warm (black circles) enclosures. Error bars show ± 1 standard error.

Table 4.10: Effect of the heat treatment on the ratio of pigments to algal biomass on the pre-colonized tiles. Results of a RM-RB-MANOVA and individual RM-RB-ANOVAs are shown. Because the *p*-value for the overall MANOVA is not significant, results for the RM-RB-ANOVAs should be compared to the more conservative Dunn-Šidák adjusted *p*-value of 0.01. (α=0.05, number of groups = 5).

Ratio	Effect	*F*-ratio	*p*
MANOVA	Heat treatment	5.70	0.10
	Time	48.7	0.0002
	Time × treatment	1.25	0.35
β-carotene: all algae	Heat treatment	4.62	0.12
	Time	3.61	0.09
	Time × treatment	0.85	0.47
Fucoxanthin: diatoms	Heat treatment	8.19	0.06
	Time	2.16	0.20
	Time × treatment	0.32	0.74
Violaxanthin: chlorophytes	Heat treatment	0.15	0.72
	Time	2.56	0.16
	Time × treatment	0.04	0.96
Chlorophyll *b*: chlorophytes	Heat treatment	0.20	0.68
	Time	0.14	0.87
	Time × treatment	0.84	0.48
Canthaxanthin: Cyanobacteria	Heat treatment	21.5	0.02
	Time	7.05	0.03
	Time × treatment	1.16	0.38

Table 4.11: Ratio of pigments (pg) to algal biomass (μg) on the STC (short-term colonized) tiles, and effect of the heat treatment on this ratio. Data shown are means from four enclosures, with standard deviations indicated in brackets. Results of a RB-MANOVA for all pigments are shown, and results of individual RB-ANOVAs are shown. Because the *p*-value for the overall MANOVA is non-significant, results for the individual RB-ANOVAs should be compared to the more conservative Dunn-Šidák adjusted *p*-value of 0.0085. (α=0.05, number of groups = 6).

	Control	Warm	*F*-ratio	*p*
MANOVA			9.17	0.06
Chlorophyll *a*: Total algae	739 (233)	320 (116)	52.0	0.005
β-carotene: Total algae	64 (36)	31 (17)	13.3	0.03
Chlorophyll *b*: Chlorophytes	488 (520)	384 (189)	$1.52 \bullet 10^{-5}$	0.997
Violaxanthin: Chlorophytes	170 (160)	64 (42)	3.51	0.16
Fucoxanthin: Diatoms	746 (497)	206 (125)	79.0	0.003
Canthaxanthin: Cyanobacteria	94 (33)	119 (36)	4.47	0.12

to chlorophyte biomass, and violaxanthin to chlorophyte biomass, although results were not statistically significant (Table 4.11). The ratio of canthaxanthin to Cyanobacteria was higher in warmed enclosures, although again, differences were not statistically significant.

Algal cell size

Although on average cell size on the pre-colonized tiles was greater in control enclosures (Figure 4.3), results were not significantly different (Table 4.12). In contrast, the average algal cell size on the STC tiles increased as a result of warming (Table 4.13). To determine whether change in the average size of individual taxa on STC tiles was driving this change, we analyzed the cell size of individual taxa. Unfortunately, because most taxa were missing from two or more enclosures, comparison was difficult. Our analyses were restricted to the five taxa that were present in a minimum of seven enclosures, *Anabaenaopsis* sp., *Nitzschia filiformis, Nitzschia sigmoidea, Achnanthes minutissima,* and *Lyngbya sp.* (Table 4.13). This criterion ensured that a maximum of one block was excluded from analysis, although by restricting analysis in this way, effects on less common species were not observable. There was no effect of the heat treatment on the average cell size of the five taxa studied (Table 4.13).

Benthic invertebrates

Cladocerans, copepods and dipterans were numerically dominant in the enclosures. The cladoceran community was dominated by *Alona sp.*, *Alonella sp.,* and *Acroperus cf. harpae* (Table 4.14). Cyclopoida was the dominant copepod order (Table 4.14). Total invertebrate abundance was unaffected by the heat treatment (Table 4.14). A multivariate analysis of the species and developmental stages present in more than two enclosures did not show a warming related shift in the community (Table 4.14). More detailed analysis showed that *Chydorus cf. brevilabris* was the only species to show a statistically significant response, doubling in abundance as a result of the heat treatment (Table 4.14). The abundance of *Ilyocryptus sp.* and *Bosmina longirostris* increased in warmed enclosures, as did *Eucyclops agilis* females and *Macrocyclops albidus* adults,

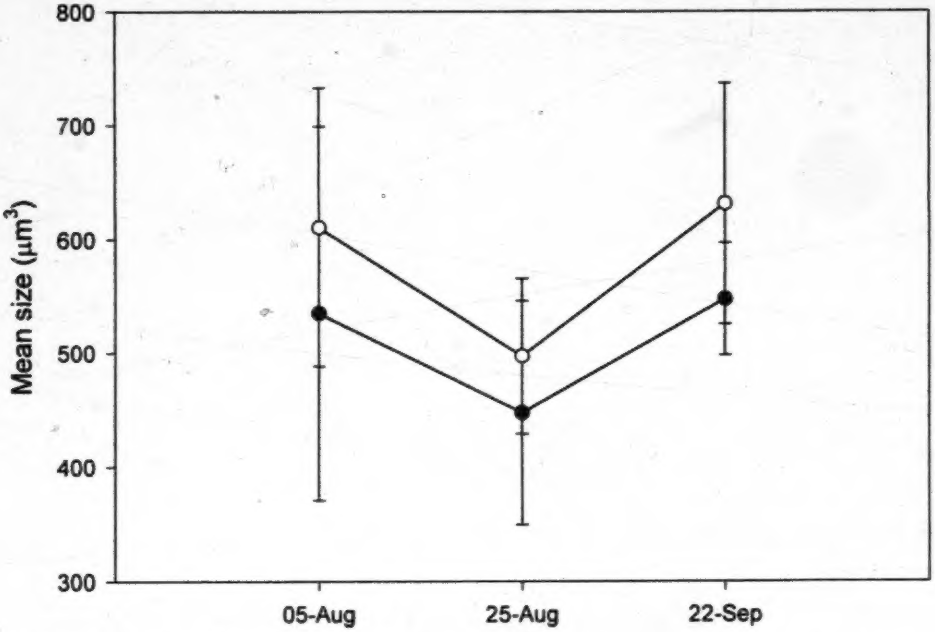

Figure 4.3: Mean algal cell size on pre-colonized tiles in control (white circles) and warm (black circles) enclosures. Error bars show ± 1 standard error.

Table 4.12: Results of RM-RB-ANOVA on the effect of the heat treatment on algal cell size on the pre-colonized tiles.

Source of variation	df	*F*	*p*
Heat treatment	1	1.63	0.29
Time	2	1.41	0.31
Time × treatment	2	0.03	0.25

Table 4.13: Effect of the heat treatment on average algal cell size on STC (short-term colonized) tiles. Mean size of all cells and mean size of the five taxa that were present in a minimum of seven enclosures are shown. Data shown are means with standard deviations indicated in brackets. RB-ANOVAs assessing the effects of the heat treatment on average algal cell size and cell size of individual taxa were performed and *F* and *p*-values for treatment effects are reported. Results of RB-ANOVAs should be compared to the more conservative Dunn-Šidák adjusted *p*-value of 0.0085 (α=0.05, number of groups = 6). Statistics were performed on the mean size of a cell from each enclosure.

	Algal cell size (μm^3)			
	Control	Warm	*F*	*p*
Average	311 (33)	934 (260)	98.5	0.002
Anabaenaopsis sp.	14.1 (0)	14.1 (0.1)	0.11	0.77
Nitzschia filiformis	247 (75)	354 (170)	0.44	0.57
Nitzshia sigmoidea	907 (236)	938 (257)	12.6	0.07
Achnanthes minutissima	92.2 (3.4)	96.4 (4.8)	1.96	0.26
Lyngbya sp.	257 (19)	253 (22)	0.10	0.77

Table 4.14: Effect of the heat treatment on invertebrate abundance. The mean abundance (m^{-2}) in four enclosures is shown with the standard deviation indicated in brackets. Copepodite stages 1-2 are indicated by c1-2, and c3-5 indicates copepodite stages 3-5. (N.d. indicates taxa were not detected.) The RB-MANOVA incorporates results for thirty- three taxa (split by gender) or developmental stages. The multivariate test is restricted to groups that were present in three or more enclosures. Individual RB-ANOVAs were also performed on groups present in three or more enclosures. The lack of significance of the overall RB-MANOVA indicates that results of ANOVAs for individual species should be compared to the more conservative Dunn-Šidák adjusted *p*-value of 0.0015 (α=0.05, number of groups = 34). Males and females of *Macrocyclops albidus* were grouped prior to analysis because females were not found in control enclosures.

Taxa	Control	Warm	*p*
MANOVA			0.43
Total invertebrates	29400 (6900)	27800 (10000)	0.81
Arthropoda			
Arachnida			
Acari	11 (22)	n.d.	
Crustacea			
total microcrustacea	14300 (5100)	15900 (6100)	0.80
Malacostraca			
Amphipoda	164 (123)	73 (24)	0.19
Brachiopoda			
Cladocera			
Chydoridae			
Acroperus cf. harpae	1210 (670)	1520 (2280)	0.72
Alona guttata	103 (145)	23 (47)	0.22
Alona quadrangularis/affinis	981 (756)	1960 (970)	0.09
Alona rustica	1900 (840)	1530 (1070)	0.72
Alona setulosa	253 (157)	1230 (1690)	0.89
Alonella cf. excisa	886 (1300)	473 (341)	0.91
Alonella nana	2070 (1920)	792 (605)	0.20
Chydorus cf. brevilabris	89 (79)	195 (68)	0.001
Chydorus gibbus	15 (30)	n.d.	
Chydorus piger	n.d.	47 (94)	
Disparalona acutirostris	87 (58)	218 (189)	0.23
Rhynchotalona falcata	n.d.	23 (47)	
Bosminidae			
Bosmina longirostris	26 (52)	320 (469)	0.002
Macrothricidae			
Ophryoxus gracilis	n.d.	27 (54)	
Streblocerus serricaudatus	n.d.	70 (140)	
Ilyocryptus sp.	213 (89)	1420 (1020)	0.004
Polyphemidae			
Polyphemus pediculus	15 (30)	95 (190)	
Daphniidae			
Scapholeberis sp.	n.d.	32 (63)	
Sididae			

Diaphanosoma birgei	15 (31)	n.d.	
Latona setifera	177 (277)	87 (94)	0.93
Sida crystallina	84 (80)	445 (477)	0.20
Ostracoda	195 (90)	220 (276)	0.35
Copepoda			
Nauplii	550 (267)	732 (532)	0.82
Harpacticoida	1240 (220)	1300 (1030)	0.60
Calanoida	37 (50)	33 (40)	0.98
Cyclopoida			
unidentified cyclopoids	22 (45)	13 (27)	
Small cyclopoids (includes all c1-2 and *Microcyclops* c3-5)	3250 (1240)	2370 (650)	0.41
Cyclopidae Sars			
Acanthocyclops cf. vernalis c3-5	524 (403)	660 (269)	0.40
Acanthocyclops cf. vernalis (female)	15 (31)	133 (129)	0.15
Acanthocyclops cf. vernalis (male)	90 (88)	93 (77)	0.95
Diacyclops bicuspidatus thomasi c3-5	15 (30)	n.d.	
Mesocyclops c3-5	18 (35)	n.d.	
Microcyclops varicans rubellus (female)	30 (59)	n.d.	
Eucyclops agilis c3-5	308 (293)	133 (104)	0.42
Eucyclops agilis (female)	41 (56)	125 (111)	0.01
Eucyclops agilis (male)	n.d.	n.d.	
Macrocyclops albidus c3-5	519 (443)	385 (222)	0.73
Macrocyclops albidus (male+female)			0.08
Macrocyclops albidus (female)	n.d.	86 (84)	
Macrocyclops albidus (male)	31 (61)	10 (20)	
Paracyclops poppei (female)	15 (30)	n.d.	
Insecta			
Trichoptera	13 (16)	13 (10)	0.66
Diptera	9920 (4670)	6620 (2550)	0.33
Small Ephemeroptera	73 (41)	91 (27)	0.58
Annelida			
unidentified annelids	78 (41)	148 (75)	0.28
Oligochaeta	4120 (650)	3950 (2070)	0.90
Cnidaria			
Hydra sp.	15 (30)	13 (27)	
Mollusca			
Gastropods	16 (6)	40 (31)	0.14

although these responses were not statistically significant (Table 4.14). Invertebrate richness and diversity were unaffected by warming (Table 4.15). The average ratio of adult copepods to stage 3-5 copepodites was greater in warmed enclosures than in controls for *Acanthocyclops cf. vernalis*, *Eucyclops agilis* and *Macrocyclops albidus*. However, once again, differences were not statistically significant (Table 4.16).

Bacteria

Average bacterial density, cell size and areal biovolume were consistently higher in warmed enclosures (on pre-colonized tiles) than in control enclosures (Figure 4.4). The increase in bacterial density was statistically significant (Table 4.17). Differences in total biovolume, and cell size were not statistically significant (Table 4.17). Increases in cell density were observed two days after the initiation of the heat treatment, and remained consistent over the course of the experiment (Figure 4.4).

Carbon

On average, carbon accumulation was slightly greater on the pre-colonized tiles in warmed enclosures than control enclosures (Figure 4.5, Table 4.18). On the STC tiles, carbon accrual was 70% higher in warmed enclosures. However, effects on both tile communities were not statistically significant (Table 4.18, Table 4.19). In contrast, carbon accrual on the natural rock substratum within enclosures was 40% greater in warmed enclosures than in controls, and differences were significantly different (Table 4.19). Carbon accrual was significantly greater on the natural bedrock substratum outside enclosures than on tiles within enclosures (Table 4.20).

Stoichiometry

Carbon to nitrogen ratios did not vary with time, or across heat treatments on pre-colonized tiles (Figure 4.6, Table 4.21). Nitrogen to phosphorus and carbon to phosphorus ratios showed a significant interaction between time and treatment effects (Figure 4.6, Table 4.21) as a result of increased carbon and nitrogen accrual in warmed enclosures on the final date (data not shown). Phosphorus accumulation was unaffected (data not shown). The heat treatment did not significantly affect nutrient ratios on the natural bedrock substratum or on the STC tiles (Table 4.22). Ratios of carbon to nitrogen, carbon to phosphorus and nitrogen to phosphorus were all significantly greater on the natural bedrock substratum outside enclosures than on tiles within enclosures (Table 4.20).

Table 4.15: Diversity (Shannon-Wiener index) and species richness of invertebrates on pre-colonized tiles in warmed and control enclosures. The Shannon-Wiener index was calculated using $\log_{10}$. Mean values of richness and diversity in four enclosures are shown, with standard deviations indicated in brackets. Results of RB-ANOVAs are also shown. Three df are associated with the each block, and 1 df is associated with the heat treatment.

	Control	Warm	*F*	*p*
Richness	26.8 (3.0)	27.5 (1.9)	1.0	0.39
Shannon-Wiener	0.79 (0.08)	0.86 (0.07)	2.4	0.22

Table 4.16: Ratio of adult copepods to stage 3-5 copepodites. Data shown are the mean ratio in four enclosures with the standard deviation indicated in brackets. Results of the multivariate test of the heat treatment on three taxa are shown. RB-ANOVAs for individual taxa are also shown. The lack of significance of the overall RB-MANOVA indicates that results of ANOVAs for individual species should be compared to the more conservative Dunn-Šidák adjusted *p*-value of 0.017 (α=0.05, number of groups = 3).

	Control	Warm	*F*	*p*
RB-MANOVA			5.4	0.10
Acanthocyclops cf. vernalis	0.26 (0.11)	0.44 (0.56)	0.54	0.51
Eucyclops agilis	0.48 (0.80)	1.00 (0.41)	3.2	0.17
Macrocyclops albidus	0.25 (0.50)	0.42 (0.43)	0.27	0.64

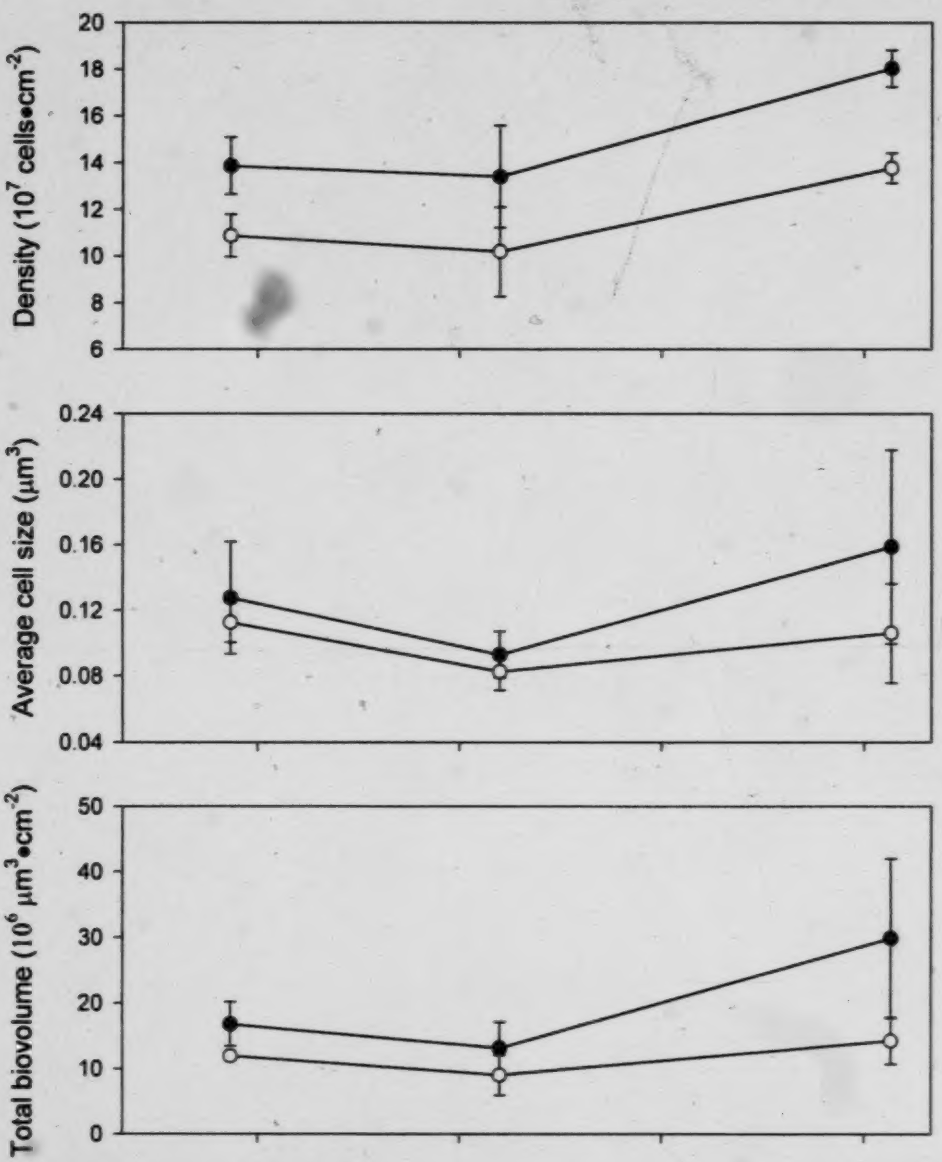

Figure 4.4: Mean density, cellular size and total biovolume of bacteria on pre-colonized tiles in control (white circles) and warm (black circles) enclosures. Error bars show ± 1 standard error.

Table 4.17: Results of RM-RB-ANOVAs on bacterial density, cellular size and total biovolume on the pre-colonized tiles. The multivariate test incorporating results for abundance, cell size and total biovolume is reported (RM-RB-MANOVA). The lack of significance of the overall RM-RB-MANOVA indicates that results of individual RM-RB-ANOVAs should be compared to the more conservative Dunn-Šidák adjusted *p*-value of 0.017 (α=0.05, number of groups = 3).

	Source of variation	df	*F*	*p*
RM-RB-MANOVA	Heat treatment	1	9.67	0.052
	Time	2	4490	$3 \bullet 10^{-13}$
	Time × treatment	2	2.09	0.20
Density	Heat treatment	1	26.8	0.01
	Time	2	30.8	0.001
	Time × treatment	2	0.71	0.53
Cell size	Heat treatment	1	2.23	0.23
	Time	2	2.34	0.18
	Time × treatment	2	0.60	0.58
Total biovolume	Heat treatment	1	6.50	0.08
	Time	2	13.4	0.01
	Time × treatment	2	0.42	0.67

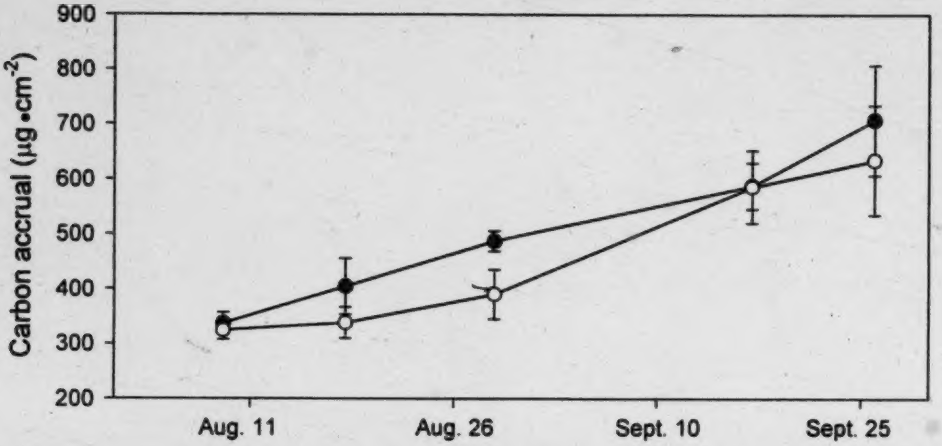

Figure 4.5: Mean areal carbon accrual in control (white circles) and warm (black circles) enclosures on pre-colonized tiles. Error bars show ± 1 standard error.

Table 4.18: Results of RM-RB-ANOVAs on areal carbon concentrations on pre-colonized tiles.

Source of Variation	df	*F*	*p*
Heat treatment	1	2.97	0.18
Time	4	67.0	$4.2 \bullet 10^{-8}$
Time × treatment	4	1.36	0.31

Table 4.19: Carbon accrual (μg cm^{-2}) on STC (short-term colonized) tiles and on the natural bedrock substratum within enclosures. Data shown are the mean of four enclosures with the standard deviation shown in brackets. Results of RB-ANOVAs are also shown. One df is associated with the heat treatment and 3 df are associated with blocks.

	Control	Warm	*F*	*p*
STC tiles	183 (36)	307 (93)	7.49	0.07
Natural bedrock substratum	1625 (262)	2306 (932)	18.9	0.02

Table 4.20: Carbon accrual (μg cm^{-2}) and molar nutrient ratios on tiles within control enclosures and the natural bedrock substratum outside enclosures on September 13, 2001. Data shown are means from four enclosures (tiles), or two sites outside enclosures (bedrock substratum). Standard deviations are indicated in brackets. One-sample *t*-tests testing the hypothesis that the mean measurement in control enclosures is equal to the mean measurement on the bedrock substratum were performed, and statistical output is shown. Three df were associated with each analysis.

	Tiles	Bedrock substratum	*t*	*p*
Carbon	586 (132)	2680 (640)	-32	$6.8 \bullet 10^{-5}$
C:N	14.8 (0.6)	15.9 (0.9)	-3.7	0.04
C:P	913 (104)	2320 (110)	-27	0.0001
N:P	61.8 (7.7)	147 (8)	-22	0.0002

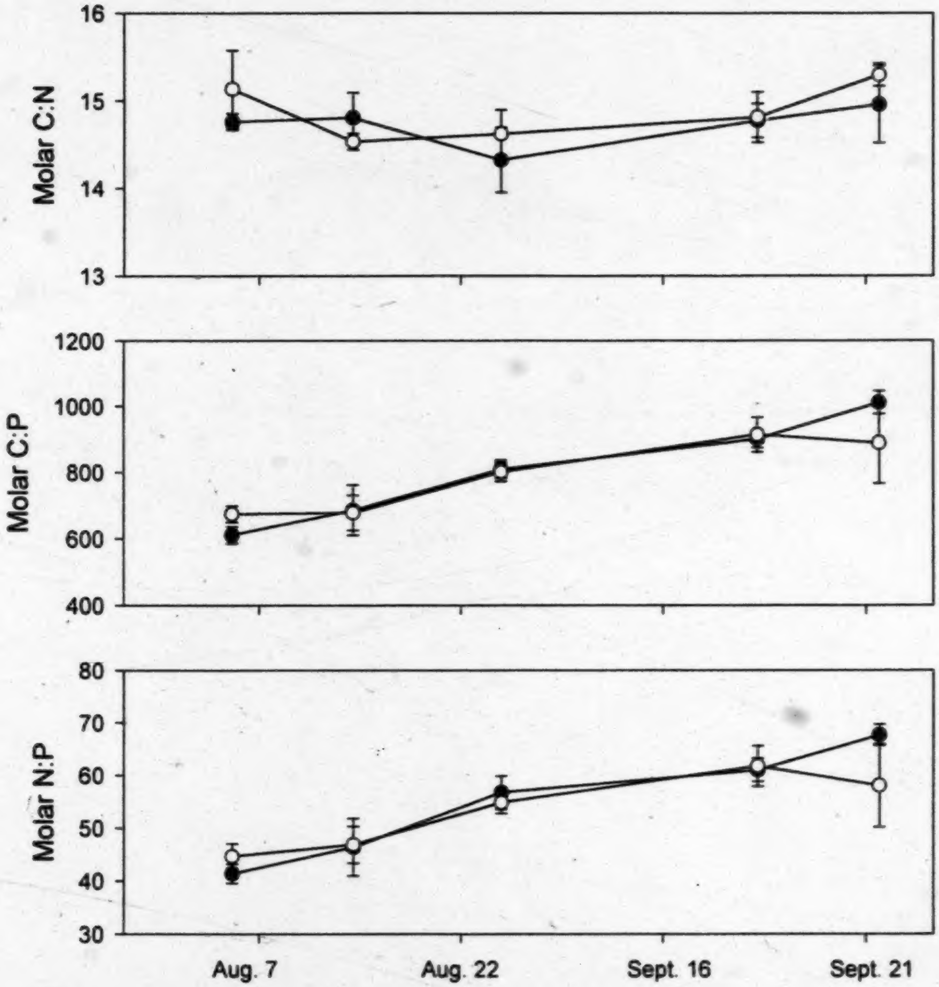

Figure 4.6: Mean molar nutrient ratios on pre-colonized tiles in control (white circles) and warm (black circles) enclosures. Error bars show ± 1 standard error.

Table 4.21: Results of RM-RB-ANOVAs on the effect of the heat treatment on epilithic nutrient ratios. The multivariate test incorporating results for all ratios is reported (RM-RB-MANOVA).

	Source of variation	df	*F*	*p*
RM-RB-MANOVA	Heat treatment	1	0.15	0.73
	Time	4	$6.85 \bullet 10^{3}$	$1.0 \bullet 10^{-15}$
	Time × treatment	4	3.56	0.04
C:P	Heat treatment	1	0.79	0.44
	Time	4	$1.3 \bullet 10^{5}$	$2.7 \bullet 10^{-6}$
	Time × treatment	4	4.46	0.02
C:N	Heat treatment	1	2.33	0.22
	Time	4	1.93	0.17
	Time × treatment	4	0.68	0.61
N:P	Heat treatment	1	1.47	0.31
	Time	4	27.7	$5.5 \bullet 10^{-6}$
	Time × treatment	4	4.54	0.02

Table 4.22: Effect of the heat treatment on molar nutrient ratios on the natural bedrock substratum within enclosures, and on the STC (short-term colonized) tiles. Data shown are the mean ratios from four enclosures with standard deviations indicated in brackets. Results of RB-MANOVAs on effect of heat treatment on nutrient ratios are also shown, and individual RB-ANOVAs are shown. One df is associated with the heat treatment and three df are associated with blocks. Because the *p*-value for the overall MANOVAs are not significant, results for the individual tests should be compared to the more conservative Dunn-Šidák adjusted *p*-value of 0.017.

Substrata	Ratio	Control	Warm	*F*	*p*
STC tiles	RB-MANOVA			0.0001	0.99
	C:P	783 (247)	765 (91)	1.59	0.72
	C:N	15.3 (1.3)	13.6 (1.5)	6.33	0.09
	N:P	51.4 (15.7)	56.4 (1.1)	0.45	0.55
Bedrock	RB-MANOVA			0.05	0.84
	C:P	1260 (240)	1320 (310)	0.05	0.84
	C:N	15.0 (0.7)	15.2 (0.5)	0.66	0.48
	N:P	84.6 (19.4)	87.2 (19.0)	0.02	0.89

Discussion

Algal taxonomy and biomass

There was no effect of the heat treatment on the biomass of different algal groups on pre-colonized tiles. This is contrary to the expectation that communities of benthic algae will shift from diatom dominance at temperatures less than 20°C to chlorophytes from 15-30°C and to Cyanobacteria at temperatures above 30°C (reviewed by DeNicola, 1996). Average temperatures ranged from 9.3-27.0°C (mean=18.0°C) within control enclosures and 13.6-31.9°C (mean=22.5°C) in warmed enclosures. Warming led to an increase in *Mougeotia* biomass on the final sampling date, although this effect was not statistically significant due to the absence of this taxon from one block of enclosures. The trend towards increased *Mougeotia* biomass in warmed enclosures is consistent with the finding in L239 that the coverage of filamentous chlorophyte algae, dominated by *Mougeotia,* is proportional to epilimnetic temperature (Schindler et al. 1990). Although we cannot dismiss the possibility that biofilm on heat exchange pipes (Chapter 2) affected study communities, we minimized effects by ensuring our study communities were separated from the pipes by at least 10 cm.

In contrast to the pre-colonized tiles, the STC tiles showed strong effects of the heat treatment on algal taxonomy. The seven-fold increase in diatom biomass was driven by a greater than twenty-fold increase in *Rhopalodia*, a large diatom that contains nitrogen-fixing endosymbionts (Rai et al., 2000). *Lyngbya* biomass doubled in warmed enclosures, driving the increase in Cyanobacteria biomass. *Mougeotia* increased in biomass, as observed for the pre-colonized tiles, but this increase was not statistically significant. *Oedogonium*, another filamentous chlorophyte, also showed interesting, although non-significant increases in biomass. The mean temperature in warmed enclosures was 22.5°C during the colonization period and in control enclosures the mean temperature was 17.6°C. These values were quite similar to the overall experimental mean temperatures. Based on DeNicola (1996), we expected that chlorophytes would begin to dominate. Contrary to DeNicola's expectations, it appears that temperatures remained within the optimal growth range for diatoms.

Biomass on the pre-colonized tiles was unaffected by the heat treatment (Chapter 3) whereas the STC tile community showed a four-fold increase in algal biomass. The increase in biomass is consistent with Gruendling's (1971) finding that seasonal changes in the biomass of epipelic algae are correlated with light and temperature. Numerous other studies have also concluded that the biomass of periphytic algae increases as a result of warming (e.g. Hickman, 1974; Eloranta, 1982). However, these studies should be interpreted with caution because conclusions were based on increases in chlorophyll *a* accrual, or ash-free dry matter (AFDM). Increases in chlorophyll *a* accrual could reflect metabolic acclimation rather than increases in biomass (Kübler and Davison, 1995; Coles and Jones, 2000), and increases in AFDM may reflect increases in the contribution of detritus, bacteria, fungi or invertebrates to total carbon.

The responses of the STC tile community and of the pre-colonized tile community may have differed for a number of reasons. If environmental conditions within enclosures differed during the four-week colonization period of the STC tiles and during the eight-week incubation period of the pre-colonized tiles, we might expect these two communities to differ. Mean water temperatures during this period were quite similar, although water chemistry differed somewhat over time (Chapter 3). Nutrient availability may have differed because the higher biomass, more mature community probably had a thicker boundary layer that limited nutrient diffusion. Alternatively, taxonomic changes may have occurred on the pre-colonized tiles; however, variability in our algal counts may have been too great to detect those changes. The STC community showed considerably less variability. The eight-week duration of the experiment may have been insufficient to induce change in an already well-developed community. STC tiles had no such community inertia. Finally, there may be some intrinsic character of newly developing communities that induces different responses to increased temperatures. For example, if algal cells on newly colonizing tiles are more metabolically active than in mature communities, as hypothesized by Barbiero (2000), responses might be observed more rapidly than within mature communities.

Effects of warming on algal diversity and species richness are contradictory. We found no effect of the heat treatment on algal diversity or species richness on pre-colonized tiles, consistent with the limited taxonomic response. The response of the STC tiles again differed from the pre-colonized tiles. A slight decrease in algal diversity was observed in warmed enclosures on the STC tiles, although richness was not significantly affected. The decrease in diversity was due in part to the dominance of *Rhopalodia* in warmed enclosures. Thermal pollution of Lake Wabamun led to reduced diversity of epiphytic algae but temperature differences were quite large, with summer water temperatures elevated by 8-9°C, and water temperatures during the winter elevated by as much as 19°C (Klarer and Hickman, 1975; Hickman 1982). Squires et al. (1979) also found that a thermal effluent depressed algal diversity on glass slides in the fall and winter months to levels usually observed only in spring and summer. In contrast, a 1977 EPA report (in Talmage and Coutant, 1979) showed an increase in the diversity of periphytic algae in a thermally polluted reach of the Missouri River. DeNicola's (1996) review suggests that algal diversity increases as temperature increases from 0-25°C. This is contrary to the observed decreases in diversity on the STC tiles in the warmed enclosures.

Algal community composition and pigment-inferred community change

Pigments are often used as indicators of algal taxonomy and algal biomass. However, we obtained contradictory results from direct algal counts and pigment analyses. Increases in the accrual of chlorophyll *a* and β-carotene, two pigments frequently used as algal biomass indicators, were not associated with increases in total algal biomass on the pre-colonized tiles. Similarly, although direct microscopic counts indicated that there was no change in the biomass of taxonomic groups on pre-colonized tiles as a result of the heat treatment, taxonomically diagnostic pigments indicated that the abundance of chlorophytes (chlorophyll *b*), diatoms (fucoxanthin) and Cyanobacteria (canthaxanthin) increased in warmed enclosures. The increased ratios of pigments to the biomass of their associated algal taxa in warmed enclosures is consistent with the observed trends, although changes were not statistically significant.

Pigment accrual on the STC tiles also showed poor agreement with taxonomic results. In contrast to results for the pre-colonized tiles, most pigments underestimated the extent of community change indicated by direct counts. However, canthaxanthin and chlorophyll *b* were good indicators of the effects of the heat treatment on Cyanobacteria and chlorophyte biomass respectively. Significant decreases in ratios of chlorophyll *a* to total algal biomass and fucoxanthin to diatom biomass were observed as a result of warming, further highlighting the discrepancy between the use of indicator pigments and direct count data. The same trend was observed for ratios of most other pigments to the biomass of their associated taxa, but changes were not statistically significant.

Although some degree of error is associated with direct algal counts, we suggest that direct counts are a more reliable metric of community change than biomass indicator pigments. Results of direct counts may be biased by counting non-viable cells, by high abundance of picoplankton-sized cells that are not observable using our taxonomic techniques, or because of difficulty associated with estimating the biomass of taxa with different geometric shapes. It is unlikely that counting non-viable cells caused the observed discrepancy, because only cells showing the presence of cellular structures were enumerated. High biomass of picoplankton-sized cells can also be ruled out, as exploratory investigation of this issue using autofluorescence techniques revealed very low abundance of small autotrophic cells. And finally, error associated with estimating algal biomass is unlikely to lead to the systematic treatment-related differences we have observed. Instead, we contend that observed changes in pigment to biomass ratios reflect metabolic acclimation to increased temperatures. Consistent with the trend observed for the pre-colonized tiles, culture studies have shown that algae may acclimate to increased temperatures by increasing their cellular pigment content (Coles and Jones, 2000). And, consistent with our observations of decreased ratios of chlorophyll *a* to total algal biomass on the STC tiles, Schindler et al. (1996), found that ratios of chlorophyll *a* to phytoplankton biomass declined during a warm, dry period at the ELA. However, these changes may also have reflected differences in light, nutrient availability or temperature.

The discrepancy between these two methods is a cautionary note to those who use pigments to infer taxonomic composition in studies of contemporary communities and paleoecological studies. Havens et al. (1999) also found poor agreement between pigment and microscope based methods for studying the taxonomic composition of epiphyton and epipelon. They suggested that variation in light, nutrient availability and species composition may have led to changes in the ratios of accessory pigments to chlorophyll *a*. Our results indicate that temperature may also affect algal pigment content, and that using pigments in habitats with a high degree of temperature variation could lead to spurious conclusions about differences in algal taxonomy and biomass.

Algal cell size

The hypothesis that increased temperatures would lead to decreased organism size (Atkinson, 1994) was not supported. There was no statistically significant effect of the heat treatment on algal cell size on the pre-colonized tiles. And, contrary to Atkinson's hypothesis, we observed an increase in algal cell size in warmed enclosures on the STC tiles. These results must be interpreted with some caution due to the variable numbers of cells that were measured. Atkinson (1994) reviewed studies where significant changes in cell size of individual species were associated with temperature changes. He found that the size of two genera of freshwater chlorophytes decreased with increased temperature, but one marine diatom species increased in size. The planktonic species discussed by Atkinson (1994) may differ in their responses from benthic species, because size related changes that may be important for buoyancy regulation of planktonic species are not advantageous to benthic species. Further, it may not be suitable to extrapolate the temperature-size relationship described by Atkinson to other taxa and across all temperature ranges. Goldman (1977) found a more complicated relationship for the planktonic marine diatom *Phaeodactylum tricornutum*, with increased biomass at high and low temperatures.

The observed increase in average algal cell size on STC tiles does not appear to reflect a change in the cell size of individual species, because none of the common species that we analyzed showed changes in mean cell size. However, we could not study changes in less common species, and as a result, chlorophytes were excluded. Atkinson found that some chlorophytes decreased in size at elevated temperatures. Instead of a change in the cell size of individual taxa, the change in average size appears to reflect a shift to larger taxa. The shift to *Rhopalodia*, a large diatom that comprised almost 5% of all cells in warmed enclosures but only 1% of cells in control enclosures contributed to this change. The average cell size of *Rhopalodia* is more than sixty times that of the average algal cell on the STC tiles. Community change therefore appears to be a more important determinant of overall average cell size than changes within a species.

Benthic invertebrates

We found that the density of benthic invertebrates, including macroinvertebrates and microcrustaceans, was unaffected by warming. This is in contrast to numerous studies that found benthic macroinvertebrate (Ferguson and Fox, 1978; Lamberti and Resh, 1983; Rempel and Carter, 1986; Hogg and Williams, 1996) and meiofauna abundance (Oden, 1979) declined as a result of warming in lakes. It is unclear why declines in invertebrates are typically observed following warming, given that increased water temperatures are associated with a lower age at first reproduction, faster egg development and increased fecundity (Abdullahi, 1990; Arnell et al., 1996). This apparent contradiction may be due to reduced longevity at increased temperatures, earlier emergence, or other factors such as food limitation or increased predation (Dusoge and Wisniewski, 1976; Abdullahi, 1990; McKee and Atkinson, 2000; Giebelhausen and Lampert, 2001).

The taxonomic composition of the invertebrate community also showed little impact of the heat treatment. *Chydorus brevilabris* doubled in abundance in warmed enclosures, and was the only taxon significantly affected by the heat treatment. These results are consistent with a study that found the growth of *Chydorus brevilabris* is positively related to temperatures, and that increased temperatures lead to a lower age at first reproduction, decreased egg development time and increased lifetime egg production of this species (Anderson et al., 1998). The trend towards increased abundance of *Ilyocryptus sp.*, *Bosmina longirostris*, *Macrocyclops albidus* adults, and *Eucyclops agilis* females in warmed enclosures may also reflect changes in physiological parameters, because the temperature dependence of development rates and reproductive rates appears to be widespread among invertebrates (Laybourn-Parry et al., 1988; reviewed in Arnell et al., 1996). However, these results were not statistically significant. The lack of effect on the abundance of other taxa was unexpected. The enclosure experiment may have been too short to observe changes in longer-lived taxa. High variability within the invertebrate community (average coefficient of variation = 110%) and low density, particularly of macroinvertebrates, may also have limited our ability to observe changes.

Oden (1979) observed much broader taxonomic changes in the meiofauna of the thermally polluted Par Pond reservoir. The abundance of ostracods, Hydracarina, nematodes and rotifers was generally lower at thermally polluted sites. Cladocerans were also affected by warming, although the effects varied over time. Increased water temperatures have been associated with increased abundance of dipterans in streams (Dusoge and Wisniewski, 1976; Ferguson and Fox, 1978), although decreases have also been shown (Hogg and Williams, 1996). Mayfly abundance may also be depressed by thermal enrichment (Ferguson and Fox, 1978) or abundance may be unaffected, as shown in this study, and by McKee and Atkinson (2000). Finally, Ferguson and Fox (1978) found that a thermal effluent in Lake Keowee, South Carolina, which increased water temperatures by a maximum of 14°C, was associated with the disappearance of Hemiptera, Odonata and Trichoptera.

Richness and diversity of benthic invertebrates were unaffected by the heat treatment. This agrees with experimental studies that showed no effect of temperature on richness of stream macroinvertebrate communities (Lamberti and Resh, 1983; Hogg and Williams, 1996) and is consistent with our findings of limited taxonomic effects. In contrast, the richness and diversity of lake macroinvertebrate communities have been reduced in thermally polluted areas (Logan and Maurer, 1975). Oden (1979) found that richness of the meiofauna community was reduced in thermally polluted areas of a reservoir, but diversity was unaffected.

Life history parameters may be more sensitive to a change in water temperatures than invertebrate abundance, richness or diversity (Hogg and Williams, 1996). Although the differences were not statistically significant, we found that the ratios of adults to stage 3-5 copepodites were greater within warmed enclosures, suggesting that development was accelerated in warmed enclosures. The response may have been confounded by continuing reproduction, although cohorts within L239 are typically discrete, and easily followed through time (M. Paterson, personal communication). Differences in survival rates of these two life stages in warm and control enclosures may have affected results. Increased water temperatures lead to reduced longevity of copepods (Abdullahi, 1990). The temperature dependence of development rates of *Acanthocyclops vernalis,* and *Macrocyclops albidus* has been established for all life stages in laboratory studies (Abdullahi and Laybourn-Parry, 1985), and similar results have been shown among other species (Sarvala, 1979).

Bacteria

Increased water temperature led to increased bacterial abundance, even during late summer when water temperatures were naturally high. Bott (1975) suggested that benthic bacteria in White Clay Creek might have been limited by temperature, rather than substrate availability. Lovell and Konopka (1985) also suggested that bacterial growth in lakes could be temperature limited. The 30% increase in bacterial abundance in warmed enclosures is similar in magnitude to a 50% increase observed in stream microcosms warmed by 8°C (Lamberti and Resh, 1983). Increased bacterial densities were also observed downstream of a thermal effluent in the Sheep River, Alberta (Osborne et al., 1983). Although the majority of bacterial cells do not show electron transport (ETS) activity, and hence are not metabolically active (Zimmermann et al., 1978; Cho et al., 1999), increased temperatures as well as an increased supply of organic substrate can induce ETS inactive cells to become ETS active (Cho et al., 1999). This suggests that changes within the bacterial community may have been even greater than observable via cell counts.

Increased substrate availability may have contributed to higher bacterial abundance. Bacterioplankton abundance in some lakes is proportional to substrate availability (del Giorgio and Scarborough, 1995), suggesting that bacteria may be substrate-limited; however, to our knowledge this has not been addressed in the benthos. Interactions between temperature and substrate availability may also occur (Felip et al., 1996). Although we did not observe an increase in algal biomass (Chapter 3) or carbon accrual on pre-colonized tiles, stimulation of DOC release by warming has been observed in cultured algae (Collins and Boylen, 1982), and could lead to increased substrate availability. Increased grazing as a result of warming (Deason, 1980; Dumont and Schorreels, 1990) could also lead to increased availability of dissolved organic matter (Møller and Nielsen, 2001). On the other hand, increased grazing would have the direct effect of reducing bacterial abundance. Increased algal biomass, as observed on the STC tiles in warmed enclosures might be expected to yield a further increase in bacterial abundance, analogous to the increase observed following a seasonal diatom bloom (Goedkoop et al., 1997).

In conjunction with the 30% increase in bacterial abundance we observed an 80-100% increase in total bacterial biovolume. However, high variability attributed to the measurement of cell size made this effect statistically non-significant (p=0.23). Temperature and bacterial production were significantly positively correlated at temperatures below 14°C in Mirror Lake bacterioplankton (Ochs et al., 1995), and bacterial growth rate and temperature were positively correlated in the seston of two rivers near Savannah, Georgia (Edwards and Meyer, 1986) and across a range of mesotrophic and eutrophic lakes (White et al., 1991). In contrast, bacterioplankton biomass has been shown to decrease at increased temperatures, although predation as well as a strong seasonal shift in average cell size may have contributed to this result (Chrzanowski et al., 1988).

Average bacterial cell size was generally greater in warmed enclosures, but differences were not statistically significant. This is contradictory to Atkinson's (1994) size-temperature hypothesis that states that temperature and average organism size are inversely correlated. Ben-Dan (2000) found that the size of planktonic bacteria increased with temperature in Lake Kinneret up to an optimum temperature of 29°C. An inverse relationship between temperature and bacterial cell size has also been observed in lake bacterioplankton (Chrzanowski et al., 1988).

Carbon accrual and stoichiometry

Increased carbon accrual may reflect increases in the biomass or carbon content of algae, bacteria or invertebrates, or increases in the detrital contribution to total epilithic carbon. Changes in photosynthesis, respiration, growth, grazing, scouring and the sedimentation of pelagic carbon could all be reflected in this metric. Carbon accrual was generally higher in warmed enclosures on the STC tiles, pre-colonized tiles and on the natural bedrock substratum; however, differences were only statistically significant on bedrock. Eloranta (1982) found that thermally polluted areas of a cooling water pond had higher dry weight, and ash-free dry weight of periphyton colonizing celluloid plates, but that average organic content was unaffected. The finding that carbon accrual on bedrock was increased as a result of warming suggests that warming will potentially lead to increased food availability for grazers, although more research is required to understand why significant increases in carbon accrual were not observed across all communities. The ecological effects of an increase in carbon availability for grazers may be minimal because epilithon in L239 is naturally carbon rich. Carbon accrual was greater on the natural bedrock substrate outside enclosures than on the pre-colonized tiles. This is likely due to increased accumulation of detritus over time.

The lack of significant effect of the heat treatment on carbon to nitrogen ratios is consistent with the findings of Goldman and Mann (1980) for two species of marine phytoplankton. Thompson et al. (1992) found a variety of responses among species of marine phytoplankton. There was a positive correlation between carbon to nitrogen ratios and temperature in two algal species. In four species, higher carbon to nitrogen ratios were observed at temperatures from 15-20°C with lower ratios at 10°C and 25°C, and no clear relationship was found in two others (Thompson et al., 1992).

Changes in non-algal components of the community may also affect nutrient ratios. However, there is a scarcity of research on temperature dependent changes in nutrient ratios of detritus, bacteria and invertebrates, and on the effect of temperature on rates of nutrient mineralization in freshwater ecosystems. Thamdrup and Fleischer (1998) found that temperature did not differentially affect rates of carbon and nitrogen mineralization in sediment from several arctic fjords and a German estuary. We should note that ratios of carbon to phosphorus, carbon to nitrogen and nitrogen to phosphorus were greater on the natural bedrock substrate outside of enclosures than on the pre-colonized tiles within enclosures, likely due to a greater accumulation of detritus that is rich in carbon, but low in phosphorus.

Nitrogen to phosphorus and carbon to phosphorus ratios showed a significant interaction between time and treatment on the pre-colonized tiles that was driven by higher ratios in warmed enclosures on the final sampling date. In contrast, the STC tiles and rock scrapings did not show a significant effect of the heat treatment on nitrogen to phosphorus or carbon to phosphorus ratios. The temporal interaction on the pre-colonized tiles is of particular interest. In culture studies of the marine chrysophyte *Monochrysis lutheri,* nitrogen to phosphorus and carbon to phosphorus ratios were highest at 19°C, intermediate at 23°C and lowest at 15°C (Goldman, 1979). If these findings are applicable to a benthic freshwater community, they suggest that these ratios should vary temporally with seasonal changes in water temperature, and that autumnal cooling could induce a different response to the heat treatment than observed at high mid-summer temperatures. Average water temperatures in the week preceding the final sampling date were 14.6°C in control enclosures and 19.3°C in warmed enclosures, corresponding to maximal differences in *M. lutheri.* The higher ratio of carbon to phosphorus in warmed enclosures at the end of the experiment indicates that food quality was degraded because invertebrates within L239 are phosphorus limited (Frost, 2001). Because this effect was driven by results of a single date and STC tiles as well as rocks showed no effect of the heat treatment, further study is required.

Conclusions

We have shown that strong community level responses may occur as a result of an increase in water temperatures within the range predicted following a doubling of atmospheric carbon dioxide. An increase in average water temperatures of 4.5°C led to significant changes in algal community composition and biomass. However, these effects were largely restricted to an early successional (STC) community. The more mature tile community showed an increase in the abundance of *Mougeotia* near the end of the experiment that was not statistically significant, although if further study demonstrates an effect, this may be biologically interesting because of the potential for blooms of filamentous green algae to affect ecosystem function (Turner et al., 1995b). Bacterial abundance also increased in warmed enclosures. Changes within the invertebrate community were minimal, and statistically significant effects on development rates were not observed during the eight-week experiment. Five invertebrate species showed interesting increases in abundance. *Chydorus brevilabris* was the only species showing statistically significant differences, although non-significant increases in the abundance of *Macrocyclops albidus* (adults), *Ilyocryptus sp., Bosmina longirostris,* and *Eucyclops agilis* (females) deserve further investigation. The reduction in food quality at the end of the experiment could have adverse effects on invertebrate grazers. Increased carbon accumulation was observed in some communities. This increase in carbon availability is unlikely to buffer the decline in food quality, because epilithic carbon is naturally abundant in L239. The complexity of responses of the study communities indicates that more research is required within different ecosystems, among different communities and at different depths to more fully understand potential warming effects on benthic communities. Longer-term studies are necessary to determine whether the changes we have observed are a harbinger of broader changes following a prolonged increase in temperature, and to determine whether changes observed at the base of the food web will have impacts upon higher trophic levels.

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Chapter 5: General Conclusions

Using long-term data analysis, we found that rates of respiration, photosynthesis and R_{dark}:P_{gross} are correlated with water temperature in L239. By developing an experimental heating system and warming enclosures, we found that metabolic rates were stimulated by warming, suggesting that temperature may be an important determinant of these rates *in situ*. We also observed an increase in R_{dark}:P_{gross}, although this effect was restricted to two dates.

Community composition changed as a result of warming. Early-successional communities (STC tiles) showed major increases in algal biomass, largely driven by an increase in diatom biomass, although the biomass of Cyanobacteria and chlorophytes also increased. Biomass of the more mature tile communities (pre-colonized tiles) showed no warming-related changes despite the stimulation of rates of net photosynthesis. And, there was no significant effect of warming on algal taxonomy of the more mature (pre-colonized) tile community. On the final sampling date, the biomass of *Mougeotia* increased on the pre-colonized tiles. This is consistent with the observation that the biomass of filamentous chlorophytes is correlated with temperature (Schindler et al., 1990; Turner et al., 1995), although our results were not statistically significant due to the absence of *Mougeotia* from one block of enclosures.

Taxonomic indicator pigments and biomass indicator pigments showed results that differed from direct counts. Increases in pigment accrual on the pre-colonized tiles suggested that chlorophyte, diatom and Cyanobacteria biomass increased as a result of warming, although no change in algal taxonomy or biomass was shown in direct count data. In contrast, several indicator pigments underestimated the extent of taxonomic change observed in direct counts on the STC tiles.

Responses of benthic invertebrates were limited. *Chydorus brevilabris* more than doubled in abundance, and there was a trend, although it was not statistically significant, towards increased abundance of *Ilyocryptus* sp., *Macrocyclops albidus* (adults), *Bosmina longirostris*, and *Eucyclops agilis* (females). Bacterial abundance increased strongly, and limited effects of warming on nutrient ratios and carbon accrual were observed.

Additional research is required to identify warming responses of natural communities and whole ecosystems, and to understand how effects of increased temperature will interact with other climate-related changes:

1. Long-term experimental studies are required to assess interactions between seasonality and warming, and to allow more complete understanding of increased temperature on longer-lived organisms. However, to fully understand whole-lake responses to warming, ecosystem-scale warming experiments are necessary (Schindler, 1998).
2. Given that controlled, ecosystem-level warming experiments are not currently feasible for lakes, long-term data analysis and large scale mesocosm warming will have to serve as surrogates. Although studies of climatic effects on lakes over the past 20 years may not be able to tell us what the next 100 years hold, long-term data analyses yield critical information that is not available from any other type of study. Larger-scale mesocosm studies can provide some of the food web complexity of long-term data analyses and whole ecosystem experimentation, but are still affected by problems of scaling and complexity (Schindler, 1998).
3. We need to determine whether prolonged warming will lead to major changes in the algal community, as observed on the STC tiles, or whether the community will remain largely unchanged, as seen on the pre-colonized tiles. Similarly, to understand responses throughout the littoral zone, we need to study responses of all benthic communities to increased temperature, and study these communities at different depths, and in lakes of differing turbulence, clarity, and nutrient status.
4. A more complete understanding of warming effects on carbon flow is necessary. This should include detailed, *in situ* studies of warming effects on invertebrate grazing rates and assimilation rates. A detailed study of warming effects on decomposition rates should also be completed.
5. Longer-term study is required to understand warming effects on invertebrates. Effects of increased water temperature may be underestimated by this study, because many taxa have life cycles lasting one year. Increasing the complexity

of the system to include fish predators is also important given that invertebrate abundance could be affected by enhanced feeding rates of fish in warmer water.

6. We need a more complete understanding of the effects of temperature on nutrient dynamics. First, we need to determine whether the observed change in nutrient ratios was a transient effect, or one that will persist through time. Second, to understand how nutrient dynamics are affected by increased water temperatures, we need to study how warming affects nutrient uptake and release and cellular nutrient quotas of benthic bacteria and algae. Some research has been done on algae; however, this research has largely been restricted to marine and planktonic species.

7. Although temperature may be one of the most important climatic effects acting upon lakes, numerous other factors will act in conjunction with increased water temperatures, including changes in light penetration (Schindler et al., 1996a, b), acidity (Yan et al., 1996), the length of the ice-free season (Robertson et al., 1992; Schindler et al., 1996a), and lake mixing (DeStasio et al., 1996). We need to better understand interactions among these and other factors at an ecosystem-scale to understand the effects of climate change on lakes. Expanding our knowledge to multiple lakes in different geographic areas is also necessary, but will be difficult, because the effects of climate change are geographically variable, and individual lakes within a geographic area may show different responses. For example, although thermocline deepening has been predicted for many lakes (Hondzo and Stefan, 1991; DeStasio et al., 1996), and was observed in L239 during a period of warm, dry weather in the 1970s and 1980s, thermocline depth in Lake Michigan is expected to decrease (McCormick, 1990). Changes in precipitation will be an important determinant of many of the physical and chemical responses of lakes to climatic change, but again, changes in precipitation are expected to be geographically variable.

8. Finally, effects of eutrophication (Chambers et al., 2001), acidification (Minns et al., 1990), overfishing (McCart, 1997; Kerr and Ryder, 1997), exotic species (Evans and Loftus, 1987; Mills et al., 1993) and accumulation of toxicants (Kidd et al., 1995) have been observed in many North American lakes. The effects of these stressors in combination with climatic change deserve study. Climatic change and eutrophication can lead to accelerated hypolimnetic oxygen depletion (Schindler et

al., 1973; Stefan et al., 1993), hence the two factors acting in concert may lead to severe problems of anoxia. However, in some lakes, climatic change may eliminate winterkill because of a decrease in the length of ice-cover, and increased under-ice photosynthesis due to reduced snow cover (Fang and Stefan, 2000). Complex interactions may be observed between toxicants and temperature. Toxicity of some chemicals increases with temperature; however, the relationship differs among chemicals (Cairns Jr., et al., 1975). Distribution of some contaminants will also be altered by changes in global temperatures due to effects on condensation and deposition of contaminants, and effects on rates of revolatilization (Wania and Mackay, 1993; Blais et al., 1998). Clearly, understanding the effects of climate change in conjunction with these stressors will be important to lake management and environmental protection in the future.

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